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# Are Pollinators Exerting Selection Pressure on the Azalea Hybrids on Gregory Bald?

Clinton Victor Kellner
*University of Tennessee - Knoxville*

To the Graduate Council:

I am submitting herewith a thesis written by Clinton Victor Kellner entitled "Are Pollinators Exerting Selection Pressure on the Azalea Hybrids on Gregory Bald?." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Ecology and Evolutionary Biology.

Edward E. C. Clebsch, Major Professor

We have read this thesis and recommend its acceptance:

Susan Riechert, Charles Pless

Accepted for the Council:
Dixie L. Thompson

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Accepted for the Council:

L Evans Roth

Vice Chancellor
Graduate Studies and Research

# ARE POLLINATORS EXERTING SELECTION PRESSURE ON THE AZALEA HYBRIDS ON GREGORY BALD?

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Clinton Victor Kellner

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## ABSTRACT

Gregory Bald is renowned for an azalea hybrid swarm which contains a very diverse array of flower colors and forms. This report involves the study of the flower constancy of the pollinators to the different azalea flower types. The flower type classification is based on the floral morphology, color and presence of odor. The azalea species involved in the hybrid swarm are Rhododendron bakeri (Lemon and McKay) Hume, R. arborescens (Pursh) Torrey and R. viscosum (L.) Torrey. Pollinators' constancy to flower type and pollinators' preference for flower type were observed on square plots that contained two or more flower types.

The following aspects of the floral biology of the plants were examined to determine if there were any differences among the flower types: protandry, wind pollination, nectar amount, percent nectar sugar, the presence of nectar amino acids, nectar constituent sugars and the effects of temperature and time on pollen and nectar availability. Pollinator morphology and activity were examined to determine the relative contribution of each species group toward the azalea seed set. The factors examined were bumblebee tongue length, pollinator foraging preference (pollen or nectar) and the effect of time and temperature on pollinator activity.

The rare flower types attracted more pollinators than the common flower types. The large number of insect visits

to the rare flower types is tenuously correlated with an increased amount of nectar sugar production of these plants. Since the rare flower types are too rare to promote flower constancy, the pollinators also visit the common flower types. Bumblebees are the most important flower visitors and cross-pollinators of the azaleas. Butterflies, small bees and vespid wasps are common flower visitors but not important cross-pollinators.

Tests for apomictic reproduction and self pollination indicate that these two forms of reproduction are not very important in this azalea population. This result combined with the observations of the flower constancy of the bumblebees indicates that outcrossing is probably the major form of reproduction of these hybrids.

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Insects

| | |
|---|---|
| Bp | *Bombus perplexus* Cresson |
| Bv | *B. vagans* Smith |
| Ba | *B. affinis* Cresson |
| Bbm | *B. bimaculatus* Cresson |
| Bi | *B. impatiens* Cresson |
| Bt | *B. terricola* Kirby |
| SS | *Epargyreus clarus clarus* Cramer, silver-spotted skipper (Hesperiidae) |

Flower Types

| | |
|---|---|
| W1-W3 | White flowers (3 types) |
| Y1-Y2 | Yellow flowers (2 types) |
| F1-F2 | Flesh-colored flowers (2 types) |
| P1-P6 | Purple flowers (6 types) |
| R1-R3 | Red flowers (3 types) |

# CHAPTER I

## INTRODUCTION

The hybrid azalea swarm on Gregory Bald in the Great Smoky Mountains National Park is world famous for its flower forms and colors. Henry Skinner (1955), an azalea enthusiast and taxonomist, gave the following description of the Gregory Bald azaleas:

> . . . thousands of plants in every imaginable hue from pure white to pale yellow, salmon yellow, clear pink and orange-red to red. Many of the flowers are yellow blotched, many of the bushes are stoloniferous and foliage varies from normal to deep glossy green, often glaucous beneath. Obviously it is a complicated hybrid swarm. . . .

These different flower colors and forms present a unique opportunity for the pollination ecologist to study flower constancy among the pollinators of the azaleas. Much can be learned about the hybridization process and this information can be compared to the formation of other azalea hybrid swarms.

To determine if the pollinators were exerting selection pressure in favor of a particular flower color or form, pollinator visitation behavior was studied in plants in several plots. The flower forms and colors in each plot were categorized into particular flower types and a record was kept of the preference of pollinators for flower type and their constancy to flower type. Differences in the floral characteristics among the flower types were correlated

with the flower type preference of the pollinators. The sugar concentrations in the nectar, nectar volume, constituent nectar sugars and nectar amino acids are all known to affect the attractiveness of the nectar to various types of pollinators. The effects these and time and temperature had on pollen and nectar availability in each flower type were examined to determine if they constituted indirect effects on the abundance of the flower visitors.

Lee (1965) reports that some azaleas reproduce by either self-pollination or apomictically. Fred Galle (personal communication) confirms that both types of reproduction occur in the azaleas that grow on Gregory Bald. I performed pollination experiments on the bald to determine the extent of these types of reproduction in various flower types because it is important to know the extent of self-pollination and apomixis to determine the importance of cross-pollination among these azaleas.

Gregory Bald is one of the many grassy balds that occurs only in the Southern Appalachian Mountains. These balds are meadows dominated by herbaceous vegetation but studded with trees and shrubs. Danthonia compressa Austin is an important component of the herbaceous vegetation and Vaccinium spp., Rubus spp., Rhododendron spp. and a scattering of trees are important components of the non-herbaceous vegetation.

The grassy balds are found on dome-shape summits, gaps

or slopes at elevations between 4,500 and 6,300 feet. Gregory Bald is located on a hilltop 4,948 feet high in the southern end of the Smoky Mountains. The slopes of Gregory Bald range from flat on top to moderate along the sides. The climate at these elevations is variable. The balds may receive 80 inches of rain a year and droughts are common in the late summer and early fall (Bruhn 1964). The weather on Gregory Bald during the bloom of the azaleas was also variable. The temperature ranged from 15°-30° C and the weather was often rainy and foggy or hot and humid.

Gregory Bald is different from the other balds because of the large number of azaleas present. These azaleas established themselves on the bald before 1925 (Carlos Campbell in Lindsay 1976). Many of these azaleas have hybridized and produced rare flower colors and forms that are not found on the other balds.

The balds are popular areas for ecological study because of their uniqueness. Many studies and speculations have been made concerning the origin of the balds, but none provides a satisfactory explanation of their origin. The trees and shrubs are presently increasing in number on the balds, and it is important that ecological studies be made of the balds before they succumb to forested successional stages.

The azalea species involved in the hybrid swarm on Gregory Bald are Rhododendron arborescens (Pursh) Torrey,

smooth azalea, R. viscosum (L.) Torrey, swamp azalea and R. bakeri (Lemon and McKay) Hume, Cumberland azalea (Fred Galle 1963 and personal communication, Li 1957). Skinner (1955) reported that a R. bakeri-like flame azalea, R. viscosum and R. arborescens are involved in the hybrid swarm. Many laymen believe that flame azalea, R. calendulaceum (Michaux) Torrey, instead of R. bakeri is involved in the hybrid swarm, but R. calendulaceum is a tetraploid (52 chromosomes) and that makes it much more difficult for it to hybridize with the other species because they are diploids (26 chromosomes). Li (1957) reports two triploid plants that are possible R. bakeri X R. calendulaceum hybrids, and Fred Galle has produced successful triploid crosses involving R. calendulaceum. Although flame azalea can hybridize with diploid azaleas, chromosome studies of the azaleas on Gregory Bald indicate that R. bakeri and not R. calendulaceum is involved in the hybrid swarm (Li 1957, Galle 1963 and personal communication).

The flower colors of these plants are variable. The flower color of R. bakeri ranges from orange, orange-red and red to lighter colors of yellow and salmon in Georgia and Alabama (Galle 1974). Both R. arborescens, smooth azalea, and R. viscosum, swamp azalea, have white flowers and often the identity of the two is confused. The red style and smooth stems of the smooth azalea distinguish this plant from swamp azalea. The smooth azalea, however, produces a

rare form that has a yellow flower. Pink forms of swamp azalea are possible hybrids with smooth azalea and hybrids of swamp azalea and flame azalea produce shades of flower colors from pink to salmon yellow (Galle 1974). Both smooth and swamp azalea produce an odor while Cumberland azalea has no scent.

Flame azalea is found in scattered localities throughout the Great Smoky Mountains National Park, and it is common very near Gregory Bald. R. bakeri is also reported in the vicinity of Gregory Bald (Li 1957). R. arborescens is a Southern Appalachian endemic and occurs on Gregory Bald as well as at lower elevations along the Little River and Little Pigeon River (Stupka 1964). In the Smoky Mountains R. viscosum occurs only on Gregory and nearby Parsons Balds (Stupka 1964). MacDonald and Coggins (1967), in contrast to Stupka, reported that they found hybrids of R. viscosum and not the pure species on Gregory Bald.

Rhododendron spp. are visited by many species of insects but many authors neglected to either identify the species of insect or the species of Rhododendron. Plath (1934) observed bumblebee habits in the eastern United States and reported that azaleas are favorite plants of queen and worker bumblebees. Speight (1967) collected Bombus impatiens Cresson on Rhododendron in East Tennessee, and Percival (1965) has collected bumblebees visiting Rhododendron in Europe. Mitchell, in his monograph of Bees of

the Eastern United States, listed bees in the genera Andrena, Nomada, Bombus, Anthophora, Megachile, Osmia, Evylaeus, Dialictus and Augochloropsis as visiting Rhododendron spp. Leppik (1974) observed bumblebees, butterflies, small bees and Doros aequalis (Loew) Syrphidae on R. nudiflorum (L.) Torrey in Maryland. Knuth (1909) reported flies, bumblebees and Lepidoptera visiting European Rhododendron spp. and Moldenke (1976), while working in California, collected honeybees, bumblebees, bee flies and small-headed flies visiting Rhododendron. Meeuse (1961) reported the White-lined Morning Sphinx and Shields (1972) listed an Indian papilionid butterfly Tres philoxenus as feeding from Rhododendron. The flowers of flame azalea and Cumberland azalea fit the description of flowers utilized by hummingbirds. (Grant and Grant 1968) and James (1948) reported that the ruby-throated hummingbird visits the flame azalea.

Previous work has been done on the azaleas on Gregory Bald. The most extensive study was undertaken by Fred Galle to study the genetics of the azaleas. He made various morphological and color measurements on the leaves and flowers of the hybrids to better understand the inheritance of the traits of the azaleas and to determine the origin of many of the hybrids. Unfortunately, after seven years of study, the only copy of his notes was stolen by a bear and the study was never completed. Robert MacDonald and A. R. Coggins (1967) collected cuttings from 32 individual azalea

clones for propagation at The University of Tennessee Arboretum and at the United States National Arboretum in Washington, D. C. They color typed the 32 clones and found quite a variety of flower colors present on the bald. They did not attempt to classify every individual flower type present. Henry Skinner, from the Morris Arboretum, extensively photographed and collected on Gregory Bald in an effort to study the azaleas taxonomically. Hui-lin Li (1957) counted the chromosomes of a few azaleas collected on Gregory Bald.

No pollination ecology research has previously been done on Gregory Bald. There is also a lack of pollination research on the Rhododendron genus in general. The diverse composition of the Gregory Bald hybrid swarm allows one to examine the attraction of flower types of different colors but similar forms to pollinators. In this study I examined aspects of the reproductive biology of the azaleas and the behavior of the pollinators that will help explain the origin of the hybrids on Gregory Bald.

In Chapter II I examine several aspects of the floral biology of the azaleas, the pollinator behavior and the nectar characteristics. In Chapter III I discuss the constancy of the pollinators to the flower types and the effect of pollinator constancy on the hybridization of these azaleas. The effect of self-pollination and apomictic reproduction on the seed set of the azaleas is discussed in Chapter IV.

# CHAPTER II

## FLORAL BIOLOGY AND POLLINATOR BEHAVIOR

Differences and similarities in the floral biology of the azalea hybrids were examined to discover possible correlations with differences in the attractiveness of the hybrids to the pollinators. Pollinator behavior was examined in order to determine if the pollinators behaved differently on different flower types. The following aspects of pollinator behavior are examined in this chapter: pollination efficiency of the flower visitors, attractiveness of the flower types, flower type preference of the potential pollinators, relative abundance of the pollinators as they visit the flower types, the behavior of the bumblebees while collecting pollen or nectar and the effect of temperature and time on pollinator activity.

The following aspects of the floral biology are discussed in this chapter: protandry and nectar availability, wind pollination, the types of flower visitors, nectar amount, percent nectar sugar, the presence of nectar amino acids, the types of nectar constituent sugars and the effects of temperature and time on nectar and pollen availability.

### I. GENERAL METHODS

I observed the pollination ecology of these azaleas from June 10 to July 9, 1977. Seed set data were collected

in late October when the capsules matured. Aspects of floral biology and nectar production were observed on selected azalea hybrids. These hybrids were classified into flower types of similar color and morphology. The plants within a flower type are presumably of similar parentage. A Royal Horticultural Society Colour Chart (Anonymous 1966) was used to classify the flower colors. Since the color varies with the age of the flower, no "old" flowers were used for the color determination. The corolla tube length and corolla diameter varied among the hybrids and some hybrids produced a detectable odor while others did not. Visitation of the flower types by pollinators was observed on a per plot basis. Twelve square plots which were 16 feet on a side were each observed for 20 minute periods. Seven of these plots were observed 10 times and five were observed 12 times. A plant was counted as in the plot if more than one-half of the crown was inside the plot boundaries.

The plots were subjectively selected because of certain combinations of flower types present in the plot. The plots were not chosen in a random manner because the majority of them would have contained solely red flowered plants. Eighty-seven of the 118 plants sampled (74%) were red flowered. Two to five flower types were usually present in the plots and the age, size and spacing of the plants varied from plot to plot. The flower type composition of

the plots also changed through time because some of the flower types finished blooming before others began to bloom.

## II. PROTANDRY AND NECTAR AVAILABILITY

### Results and Discussion

Azaleas are protandrous (the anthers begin to shed their pollen before the stigma becomes receptive). The pores in the anthers mature before the flower bud opens in pinxter-flower, Rhododendron nudiflorum, flame azalea and the hybrids on Gregory Bald. Bees (Bombus spp., Andrena spp. and Halictidae) often enter the bud to collect pollen. The pollen tetrads are held together by viscin threads which aid in the dispersal of the pollen by flower visitors. As the insect's body touches the pore the viscin threads stick to the insect and a string of pollen is withdrawn. Faegri and van der Pijl (1971) report that in some Rhododendron species the anther contracts and the pollen is squeezed from the anther. This does not happen in these hybrids. Although pollen is mature while the flower is in bud, cleistogamy (self-pollination within a closed flower bud) does not occur because the stigmas are spatially separated from the anthers.

The stigmas became receptive one or two days after the flower bud opened (Table I). This characteristic was examined for the flower types because the length of time before the

stigmas become receptive could influence the seed set of the flower types.

TABLE I

THE NUMBER OF DAYS UNTIL STIGMA RECEPTIVITY

| Flower Type | P-1 | R-1 | R-2,R-3 | W-3 |
|---|---|---|---|---|
| Plant 1 | 1 | 1 or 2 | 1 | 1 or 2 |
| Plant 2 | 1 or 2 | | 1 | |
| Plant 3 | | | 1 or 2 | |
| Plant 4 | | | 1 or 2 | |

Determining stigma receptivity was a difficult task and was often a subjective determination. Stigma morphology, color and stickiness differ between receptive and unreceptive stigmas. The color and stickiness also vary from flower type to flower type. In general the stigmas of red, flesh and yellow flower types change from dry and green to sticky and lighter green. Sometimes the stigmas may be sticky when the flower bud opens and become more sticky when receptive. The white and purple flower types have purple stigmas that are dry or sticky when unreceptive or stickier when receptive. The stigmas of all the flower types also enlarge and the stigmatic surface becomes smoother than before. A stigma

past receptivity turns whitish and pollen does not adhere to it as well. These changes are continuous and it is difficult to determine exactly when the stigma becomes receptive or past receptivity.

In contrast to pollen, nectar is not available throughout the flowering period of the plants. While the flower is in bud and during the first 24 hours that the flower is open, no nectar is produced. The beginning of nectar production coincides with the ripening of the stigma. This may indicate that the plant is adapted to pollination by a nectar gatherer. The nectar is available throughout the rest of the flowering period until shortly before the corolla detaches from the receptacle.

Lee (1965) noticed that unpollinated flowers remain on the plants longer than pollinated flowers. The flowers I observed inside the wire insect excluders did the same thing. This appears to be an adaptation to increase the chances of pollination in flowers that have not been pollinated.

In the next sections the effect of wind and insects as agents of pollen dispersal is discussed.

## III. WIND POLLINATION

### Methods and Results

Wind does not seem to be a very important factor in the pollination of these azaleas. One hundred and sixty-one flowers placed inside cylindrical insect excluders 16

centimeters in diameter and 48 centimeters long fabricated from one-quarter inch hardware cloth had a low seed set (Table II). Wind was freely able to blow through the enclosures and insects seldom entered them. Only 13 capsules were produced from 161 flowers (eight of which were probably bumblebee pollinated). In light of the low seed set, the bias introduced by restricting the branch movement of the enclosed flowers is minimal.

## Discussion

Fred Galle (personal communication) has observed cases of wind pollination in some azaleas. In those plants the wind blew the stamens so that they touched the stigmas and styles of nearby flowers and hence effected pollen transfer. Viscin threads prevented the pollen from being blown from the anthers as it is in most wind pollinated plants. The wind usually effected self-pollination, but might have caused cross-pollination where azalea plants grew so close together that they touched each other. During and after wind pollination the flowers appear cobwebby because the viscin threads are draped about the flowers. This was not observed on Gregory Bald.

A factor that inhibits wind pollination on Gregory Bald is that the bees often collect the pollen shortly after the flower opens and no pollen is left for wind pollination. In the subsequent section the types of pollinators are examined.

TABLE II

SEED SET IN THE WIRE FLOWER ENCLOSURES

| Flower Type | Plant | Number of Flowers Enclosed | Number of Capsules Formed | Number of Seeds Produced |
|---|---|---|---|---|
| W-2 | 1 | 7 | 1 | 6 |
| W-2 | 2 | 16 | 0 | 0 |
| W-3 | 1 | 3 | 0 | 0 |
| W-3 | 2 | 5 | 0 | 0 |
| F-2 | 1 | 4 | 0 | 0 |
| F-2 | 2 | 41 | 8 | 26,7,19,34,31,28, 21 (Bumblebee inside excluder)* |
| F-2 | 3 | 6 | 0 | 0 |
| P-1 | 1 | 11 | 0 | 0 |
| P-1 | 2 | 8 | 0 | 0 |
| P-2 | 1 | 6 | 0 | 0 (Bumblebee inside excluder) |
| R-1 | 1 | 7 | 0 | 0 |
| R-1 | 2 | 5 | 1 | 26 (capsule mostly eaten)** |
| R-3 | 1 | 7 | 0 | 0 (Bumblebee inside excluder) |
| R-3 | 2 | 10 | 0 | 0 |
| R-3 | 3 | 5 | 3 | ? |
| R-3 | 4 | 5 | 0 | 0 |
| R-2,R-3 | 1 | 4 | 0 | 0 |
| R-2,R-3 | 2 | 4 | 0 | 0 |
| R-2,R-3 | 3 | ? | 0 | 0 |
| | | 161 | 13 | 203 |

*A bumblebee was observed inside the insect excluder.

**A coleopterous or lepidopterous larva devoured the capsule contents before the seeds could be counted.

## IV. THE TYPES OF FLOWER VISITORS

Rhododendron species attract many different kinds of potential pollinators. Moldenke's (1976) and Mitchell's (1960, 1962) lists demonstrate that single species of Rhododendron or groups of closely related species attract a wide variety of flower visitors. I observed bumblebees, honeybees, small bees, butterflies, flies, wasps, beetles, moths and the ruby-throated hummingbird visiting the flowers of the Gregory Bald azaleas (Table III). The morphology of these azalea flowers (a long slender corolla tube and exerted stamen and style) suggests a specific relationship with a particular pollinator species or type of pollinator (Faegri and van der Pijl 1971). It is surprising that a wide variety of pollinators visited the azaleas on Gregory Bald.

Not all of these flower visitors are equally efficient at transferring pollen from anther to stigma. In the next section I discuss the pollination efficiency of the flower visitors.

## V. POLLINATION EFFICIENCY OF THE FLOWER VISITORS

A visit to a flower type does not imply that pollination has occurred. Many flower visitors of the azaleas are not pollinators. Some of these flower visitors visit the flowers for pollen, others seek nectar and some seek both

TABLE III

POLLINATOR IMPORTANCE VALUES*

| Taxon | The Relative Abundance** of the Species of Flower Visitors | | | Efficiency | I.V. |
|---|---|---|---|---|---|
| | Order | Family | Species | | |
| INSECTA | | | | | |
| Hymenoptera | 90.2 | | | | |
| Apidae | | 70.6 | | 0.33 | 23.3 |
| Bombus perplexus Cresson | | | 19.3 | | |
| B. vagans Smith | | | 10.9 | 0.33 | 6.4 |
| B. bimaculatus Cresson | | | 13.0 | 0.33 | 4.3 |
| B. affinis Cresson | | | 8.2 | 0.33 | 2.7 |
| B. impatiens Cresson | | | 16.2 | 0.33 | 5.3 |
| B. terricola Kirby | | | 2.2 | 0.33 | 0.7 |
| Apis mellifera L. | | | 0.6 | 0.33 | 0.2 |
| Andrenidae | | 6.3 | | | |
| Adrena spp. | | | 6.3 | 0.2 | 1.3 |
| Halictidae | | 2.4 | | 0.2 | 0.5 |
| Augochlora pura Say | | | + | | |
| Augochlorella striata Provanchier | | | + | | |
| Augochloropsis metallica Fabricius | | | + | | |
| Evylaeus quebecensis Crawford | | | + | | |
| Dialictus laevissimus Smith | | | + | | |
| D. abanci Crawford | | | + | | |
| D. versans Lovell | | | + | | |
| D. imitatus Smith | | | + | | |
| D. caeruleus Robertson | | | + | | |

TABLE III (continued)

| Taxon | The Relative Abundance** of the Species of Flower Visitors | | | Efficiency | I.V. |
|---|---|---|---|---|---|
| | Order | Family | Species | | |
| Xylocopid e | | 0.1 | | | |
| Xylocopa virginica L. | | | 0.1 | 0.33 | 0.03 |
| Anthophoridae | | 0.1 | | | |
| Nomada sp. | | | 0.1 | 0.2 | 0.02 |
| Vespidae | | 10.4 | | | 1.0 |
| Vespula norwegicoides Sladen | | | 6.2 | 0.1 | 0.6 |
| V. artica | | | 2.8 | 0.1 | 0.3 |
| V. arenaria Fabricius | | | 0.6 | 0.1 | 0.06 |
| Eumenes spp. | | | 0.2 | | |
| Formicidae | | | | | |
| Formica subsericea Say | | | + | | |
| Ichneumonidae | | 0.1 | | | |
| Netelia sp. | | | 0.1 | | |
| Lepidoptera | 6.5 | | | | |
| Hesperiidae | | 6.0 | | | |
| Epargyreus clarus clarus Cramer | | | 5.8 | 0.2 | 1.2 |
| Erynnis sp. | | | 0.2 | 0.1 | 0.02 |
| Papilionidae | | 0.2 | | | |
| Papilio glaucus L. | | | 0.2 | 0.5 | 0.1 |
| Craphinum sp. | | | + | | |
| Daneidae | | | | | |
| Danus plexippus plexippus L. | | | + | | |
| Lycaenidae | | 0.2 | | | |
| Unknown | | | 0.2 | 0.05 | 0.01 |
| Sphingidae | | | + | | |
| Unknown | | | | | |

TABLE III (continued)

| Taxon | The Relative Abundance** of the Species of Flower Visitors | | | Efficiency | I.V. |
|---|---|---|---|---|---|
| | Order | Family | Species | | |
| Noctuidae | | | | | |
| Autographa biloba (Stephens) | | | + | 0.5 | |
| Heptagrotis phyllophora (Grt.) | | | + | 0.2 | |
| Geometridae | | | | | |
| Unknown | | | + | | |
| Unknown Lepidoptera | | | 0.1 | 0.05 | 0.005 |
| Diptera | 2.3 | | | | |
| Syrphidae | | 1.7 | | | |
| Eristalis tenax L. | | | 0.9 | 0.05 | 0.05 |
| Allograpta sp. | | | 0.8 | 0.2 | 0.16 |
| Tachinidae | | 0.5 | | | |
| Epalpus signifer Wlk. | | | 0.5 | 0.5 | 0.03 |
| Calliphoridae | | 0.1 | | | |
| Unknown | | | 0.1 | 0.05 | 0.005 |
| Coleoptera | 0.3 | | | | |
| Cerambycidae | | 0.1 | | 0.1 | 0.01 |
| Pidonia densicolla Say | | | + | | |
| P. aurata Korn | | | + | | |
| P. ruficollis Say | | | + | | |
| Elateridae | | 0.1 | | | |
| Amphedus sp. | | | 0.1 | >0.05 | >0.005 |
| Cantheridae | | | | | |
| Cantharis sp. | | | + | | |
| Scarabaeidae | | 0.1 | | | |
| Popillia japonica Newman | | | 0.1 | 0.05 | 0.005 |

TABLE III (continued)

| Taxon | The Relative Abundance** of the Species of Flower Visitors | | | Efficiency | I.V. |
|---|---|---|---|---|---|
| | Order | Family | Species | | |
| Orthoptera | | | | | |
| Acrididae | | | + | | |
| AVES | | | | | |
| Trochilidae | | 1.0 | | | |
| Archilochus colubris | | | 1.0 | 0.5 | 0.5 |

*Importance values (I.V.) were calculated by multiplying the relative abundance times the efficiency.

**The relative abundance was calculated as a percent.

+Flower visitors that were observed on the plants that were not in the plots.

pollen and nectar. The following discussion compares the pollination efficiencies of the more common flower visitors. Table III presents pollination importance values for many of these potential pollinators. These values were calculated by multiplying the relative abundance of the pollinators by the efficiency of the pollinators (the number of times the pollinator touched a stigma per flower visit). The relative abundance is a percentage that is calculated from the visitation to the plots by the pollinators. The number of times the pollinators touched the stigma per flower visit was a subjective determination based on observation of the pollinators in the plots.

Results and Discussion

The bumblebee workers are the most frequent visitors to the azalea flowers, but they are not very efficient at pollen transfer. The nectar gatherers are inadvertent pollinators because they seldom touch the stigma. The stigmas are exerted far beyond the corolla opening and the bumblebees do not touch the stigmas while resting on the corolla and probing the corolla tube. Occasionally while approaching the flower, the worker will bump into the stigma.

Queen bumblebee nectar gatherers can be effective pollinators of the flowers because of their larger size and because of the way they approach the flowers. A queen approaches the flowers from the front or from the side.

If she approaches from the front, then she has a chance of pollinating the flower by flying into the stigma and anthers. Sometimes she will walk from flower to flower in the umbel and not effect much pollen transfer.

Bumblebees that collect pollen are more efficient pollinators than bumblebees that forage nectar. Often the style was exerted above the filaments but when the stigma was near the anthers the bumblebees touched the stigma while gathering pollen. Although the bumblebees touched the stigmas they did not carry much pollen on their bodies and they did not effect much pollination if considered on a per flower visit basis. Nevertheless, the pollen collecting bumblebees were probably the single most important pollinator group in terms of numbers of individuals and amount of pollination effected although they were inefficient pollinators.

The bumblebees used two different foraging methods to gather pollen. They either hovered over the anthers and scrabbled them for pollen or landed on the filaments and removed the pollen with their mandibles or maxillae. They held onto the filaments with their second and third pairs of legs and transferred the pollen to the corbicula (pollen basket) with their front legs. Both types of pollen collecting behavior were used by all six species of bumblebees.

Butterflies were common flower visitors to the azaleas. The silver-spotted skipper, Epargyreus clarus clarus

Cramer, was the most common butterfly on Gregory Bald. These butterflies land on the corolla and probe the corolla tube from the side. Leppik (1974) also observed butterflies probe the corolla tube from the side, but he observed butterflies covered with pollen. I saw no butterflies covered with pollen, but I occasionally saw butterflies touch the anthers and stigmas. The wing tips touched the anthers and stigma as the butterfly probed the corolla for nectar. The tiger swallowtail, Papilio glaucus L., an uncommon flower visitor, behaves like the silver-spotted skipper when visiting flowers. It has a greater chance of touching the stigmas than does the skipper because its wings are larger. It may not only touch the reproductive parts of the flower it is resting upon, but I observed it touch those on nearby flowers as well.

The effect of small insect flower visitors (small bees and flies, wasps, ants and cerambycid beetles) on the pollination of the azaleas was tested by using cylindrical wire insect excluders 16 centimeters in diameter and 48 centimeters long fabricated from one-quarter inch hardware cloth. The small flower visitors rarely entered these flower enclosures, but a few bumblebees entered these enclosures and in one case may have effected some pollination (Table II, page 14). The wire excluders may be most useful in a pollination study of R. nudiflorum because the smaller insects are more abundant (Leppik 1974 and personal observations).

The small bees are fairly common flower visitors, but they rarely touched the stigma. While collecting pollen they landed on the corolla, filaments or style and crawled to the anthers or they landed on the anthers. During pollen collection the bees held onto the bottom of the anther or the filament with their hind legs, extracted the pollen with their mouthparts and then, using their first two pairs of legs, transferred it to their hind legs. While collecting nectar the small bees stayed on the corolla surface and did not crawl up the stamen. They probed the corolla tube or crawled to the base of the detached corolla and probed for nectar.

Vespid wasps (Vespula spp.) were also common visitors to the azalea flowers. These wasps foraged solely for nectar and rarely touched the reproductive parts of the flower. The hairy bodies of these wasps picked up a few pollen grains but they rarely touched the stigmas. Their nectar-foraging behavior was much the same as that of the small bees. They confined their visits to the corolla and probed either the open corolla tube or the detached corolla base of the flower.

The flies that are attracted to the flowers are also not efficient pollinators of the plants. Eristalis tenax (L.) (Syrphidae) and Epalpus signifer (Wlk) (Tachinidae) were nectar feeders that confined their flower visits to the corolla. They do not touch the stigmas nor do they

crawl to the base of the detached corolla as the small bees and wasps do. Allograpta probably obliqua (Dr. Vockeroth personal communication) (Syrphidae) is a pollen feeder and occasionally will touch the stigma. Allograpta is a small fly that usually hovers and lands on the anthers and probes them with its proboscis. It will also probe the stigma although it probably does not effect much pollination.

Ants and cerambycid beetles were also found on the flowers but they probably effected little pollination. The ants, Formica subsericea (Say), crawled about the corolla surface and probed the corolla tube for nectar. They probably stayed on one plant and did not effect any cross-pollination. The beetles (three species of Pidonia) fed on the pollen. They flew to the flowers and landed on the corolla, the filaments or the style and then climbed up to the anthers. Although pollen stuck to the beetles and the beetles occasionally touched the stigmas they effected little pollination.

The ruby-throated hummingbird, Archilochus colubris, was an infrequent flower visitor. The hummingbirds I encountered were nervous in my presence, which made them less likely to visit the flowers within the plots. The hummingbirds I was able to observe touched the stigmas and anthers a greater percentage of the time than did the butterflies or bumblebees. Pollen was observed to stick to the head, throat and back of the bird while it was

visiting flowers. The relationship between the morphology of the hummingbird and the azalea flower was not perfect because the stamen and style curved away from the path of the hummingbird. Often the ruby-throat avoided the stamen and stigma. It is not known if this was intentional avoidance or accidental behavior. The flowers adapted for hummingbird pollination most often have red coloration and long tubular corollas with exerted stamen and styles (Grant 1966). Baker (1961) reports that hummingbirds are not flower constant unless there is a large group of a single flower species present. Bené (1941) observed that the black-chinned hummingbird exhibited no color preference to solutions of colored sugar water. The hummingbirds on Gregory Bald did not show a color preference for red flowers and they were not constant to any flower type.

Sphingid moths were rare visitors to the flowers but they probably are effective pollinators. They hover while flower visiting in such a position that they touch the stigmas and anthers with the underside of their abdomens or thoraxes. Their proboscises are long enough that they do not have to rest on the flowers while gathering nectar. Hemaris diffinis (Boisduval), the bumblebee hawkmoth, a day-flying sphingid, was a common visitor to the flowers of R. nudiflorum. The pollination relationship between the hawkmoth and Rhododendron seemed to be efficient; this hawkmoth touched the stigmas on almost

every flower it visited. Other hawkmoths probably behave the same way as Hemaris on azalea flowers.

Other species of nighttime flower visitors included two species of Noctuidae and one species of Geometridae. One of the noctuids, Autographa biloba (Stephens) hovered over the flowers while foraging for nectar and probably effected some pollination although it is smaller than the sphinx moth and has a shorter proboscis. The other noctuid species, Heptagrotis phyllophora (Grt.), did not hover, but crawled from flower to flower in the umbel. Because this species crawled from flower to flower it probably effected less pollination than Autographa although both species had a few pollen grains on their bodies. The Geometrid species (unidentified) tended to crawl from flower to flower but more observations are necessary to determine if this is the case.

All of these moths were only observed on two separate clones of one flower type (P-1) and an isolated Rhododendron maximum L. Flower type P-1 has light colored, odoriferous flowers with long tubular corollas. These characteristics fit the description of "typical" moth pollinated flowers (Baker 1961). There were many other azalea plants that fit the description of "moth pollinated flowers" that received no visits from moths. No reason is proposed for the selectiveness of these moths.

In the preceding sections wind and insects were

discussed as agents of pollen dispersal. Insects and hummingbirds and not wind effect the pollination of the azaleas. Among the flower visitors the bumblebees are probably the most important pollen vectors, though hummingbirds and moths may be more important than indicated by this study. In the following section the attraction to flower types and preference for flower types by pollinators are examined.

## VI. ATTRACTIVENESS OF THE FLOWER TYPES, FLOWER TYPE PREFERENCE OF POTENTIAL POLLINATORS AND THE RELATIVE ABUNDANCE OF THE POLLINATORS AS THEY VISIT THE FLOWER TYPES

Casual observations of the plants on Gregory Bald indicate that certain plants attract many more pollinators than other plants. An attractiveness index was calculated to show which flower types attract the most pollinators. The values are calculated on a "per plant" basis. The flower type preference of the potential pollinator species was examined to determine if certain pollinators were attracted to certain flower types more than others. A flower type preference exhibited by a pollinator group may affect gene flow among the azaleas on the bald. The relative abundance of the pollinators is defined to be the rank order of the flower visitor species that visited a certain flower type (the most frequent visitor species was

given rank 1, the second most frequent species rank 2, etc.). A comparison of the rank orders of the flower visitors between the flower types might indicate which flower types are the most similar from the point of view of the pollinators.

Methods

During the 20-minute plot sampling period, the arrival of each potential pollinator was recorded, as was the first flower type to be visited. A potential pollinator touched the flower for a "visit" to be scored; hovering about the plant or landing on a leaf did not count as a "visit."

Results: Attractiveness of the Flower Types

The attractiveness index (Table IV) was computed by dividing the total number of observed visits to a flower type from all potential pollinators by the total number of times every plant of that flower type was sampled in the plots. This index compensates for the differences in abundance of the flower types. For instance, R-3 was the most common flower type (376, Table IV), and it received the most total flower visits (267), but in comparison to its abundance, it was not visited as much (0.7) as the more attractive flower types. The attractiveness of the individual plants of a given flower type varied greatly depending on the size of the plant, the position of the

TABLE IV

THE INDEX OF ATTRACTIVENESS OF THE FLOWER TYPES*

| Items | Flower Type | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | F-1 | P-5 | P-1 | W-2 | W-3 | P-3 | F-2 | P-2/4/6 | R-1 | R-3 | Y-2 | R-2 |
| Number of Plants | 1 | 2 | 3 | 3 | 5 | 5 | 4 | 6 | 11 | 47 | 3 | 29 |
| Number of Visits to a Flower Type | 77 | 56 | 135 | 64 | 90 | 65 | 135 | 45 | 84 | 267 | 16 | 141 |
| Sample Size | 13 | 12 | 35 | 21 | 40 | 33 | 71 | 41 | 93 | 376 | 23 | 227 |
| Attractiveness Index | 5.9 | 4.7 | 3.9 | 3.0 | 2.3 | 2.0 | 1.9 | 1.1 | 0.9 | 0.7 | 0.69 | 0.6 |

*This index was calculated by dividing the total number of visitors to a flower type by the number of times that all plants of that flower type were observed for visitation (sample size).

plant with respect to other azaleas or other vegetation and the age of the flowers on the plants. A large number of plants sampled within a flower type provides a better indication of the attractiveness of that type because the attractiveness of the flower types varies among plants within flower type. Since some of the flower types are represented by only one or two plants, the attractiveness index is a good qualitative rather than a quantitative tool. Nevertheless, this index shows that the more common red flowered plants are less frequently visited and are, therefore, less attractive than the rare white, purple and flesh-colored flower types.

Results: Flower Type Preference of Potential Pollinators

From the record of the flower type first visited by each species and from the record of the density of shrubs with various flower types within plots, it was possible to calculate the expected visitation of each flower type by the potential pollinators. The expected visitation values were calculated from the probability that a flower type would receive a visit. These probabilities were calculated from the density of the flower types each time a visit occurred to a plot. For instance, in a plot containing four red flowered plants and one white flowered plant, the expected probability that the red flower type receive a visit is 4/5 and the white flower type is 1/5 for every visit that occurs to that plot.

The data are presented in Appendix II (Tables XXVII and XXVIII). A significant result of the G-test (P=.05) indicates that certain pollinator species prefer certain flower types. Tables V and VI show the number of observed visits per expected visit. Numbers near one indicate that the observed number of visits was near the expected number of visits. Values greater than one indicate that a flower type was preferred by pollinators, and values less than one indicate that pollinators were not attracted to a particular flower type.

Chi square analysis of the cells of Appendix II, Tables XXVII and XXVIII, are presented in + and - form in Table VII. This table shows trends in the attractiveness and avoidance of flower types by insects. The data in Table VII show that most insects avoid the red flower types or visit them about as expected. Some purple types are avoided by most species (or visited by them about as expected), but others are preferred. The white and flesh types generally attract many species of insects or the data are inconclusive or visitation is about as expected. This table also shows that the flower types with the larger attractiveness indices (Table IV) are generally the flower types preferred by the potential pollinators.

Floral morphology does not seem to play a part in determining preference of flower types by pollinators. The red flower types have short corollas (Appendix I,

TABLE V

THE NUMBER OF OBSERVED VISITS PER EXPECTED VISIT TO THE FLOWER TYPES BY THE BUMBLEBEES

| Insect | Flower Type | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | W-2 | W-3 | F-1 | F-2 | Y-2 | P-1 | P-2/4/6 | P-3 | P-5 | R-1 | R-2 | R-3 |
| B. perplexus | 2.04 | 4.48 | 0.75 | 0.64 | 0.25 | 1.29 | 0.83 | 1.50 | 1.90 | 1.30 | 0.66 | 0.92 |
| B. vagans | 1.67 | 2.73 | 2.40 | 0.53 | 0.74 | 0.83 | 0.13 | 0.17 | 1.67 | 1.53 | 0.69 | 1.00 |
| B. affinis | 2.12 | 2.00 | 0.91 | 0.80 | 0.00 | 2.09 | 0.50 | 1.20 | 2.27 | 0.27 | 0.69 | 1.09 |
| B. bimaculatus | 3.30 | 3.30 | 2.20 | 0.77 | 1.48 | 1.83 | 0.28 | 0.83 | 2.11 | 0.57 | 0.60 | 0.96 |
| B. impatiens | 2.30 | 5.65 | 2.27 | 1.24 | 1.82 | 1.64 | 0.41 | 1.85 | 1.15 | 0.32 | 0.53 | 0.63 |

TABLE VI

THE NUMBER OF OBSERVED VISITS PER EXPECTED VISIT TO THE FLOWER TYPES BY THE OTHER POLLINATORS

| Insect | Flower Type | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | W-2/3 | F-1/2 | P-1 | P-2/4/6 | P-3/5 | R-1 | R-2 | R-3 |
| Silver-spotted skipper | 5.15 | 0.48 | 3.39 | 0.13 | 2.00 | 0.21 | 0.42 | 0.60 |
| Small Bees | 3.30 | 0.83 | 1.70 | 0.36 | 1.27 | 0.93 | 0.74 | 0.99 |
| Vespula spp. | 2.50 | 0.93 | 1.20 | 0.52 | 1.06 | 0.81 | 0.31 | 1.13 |
| Other | 4.12 | 0.85 | 2.90 | 0.00 | 1.67 | 1.00 | 0.51 | 0.54 |

TABLE VII

FLOWER TYPE PREFERENCE OF THE POLLINATOR SPECIES

| Insect | Flower Type | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | W-2 | W-3 | F-1 | F-2 | Y-2 | P-2/4/6 | P-1 | P-3 | P-5 | R-1 | R-2 | R-3 |
| B. perplexus | + | + | | | - | | | | + | | - | |
| B. vagans | | + | + | | | - | | | | | | |
| B. affinis | ? | ? | | | | | + | | + | - | | |
| B. bimaculatus | + | + | + | | | - | + | | + | | - | |
| B. impatiens | + | + | + | | | - | + | + | | - | - | - |
| B. terricola | | ? | ? | | | | | ? | | | | - |
| Silver-spotted skipper | ? | + | | | | - | + | | | - | - | |
| Small Bees | | + | | | | - | + | | ? | | | |
| Vespula spp. | ? | ? | + | + | | | | | | | - | |

+ indicates a significant (chi square) attraction toward a flower type; - indicates a significant (chi square) aversion of a flower type; ? indicates a possible attraction toward a flower type but the expected values were too low to be statistically sure; blank values indicate that the observed values were not significantly different from the expected values.

Table XXVI), but they do not differentially attract the short tongued bumblebees Bombus affinis Cresson and B. terricola Kirby. [Medler (1962) presents data on the tongue lengths of many species of bumblebees.] Conversely, the longer tongued bumblebees, B. vagans Smith and B. bimaculatus Cresson, and the silver-spotted skipper, Epargyreus clarus clarus Cramer, are not solely restricted to foraging on the flower types with long corollas. Corolla length, therefore, does not affect the flower type preference of the pollinators.

Results: Relative Abundance of the Pollinators as They Visit the Flower Types

I used Spearman rank correlations (Snedecor and Cochran 1967) to determine if the rank order of the abundance of the pollinator species that visited a flower type was similar for any two flower types (Table VIII). There are correlations between similar flower types (F1, F2; P1, P3) and between dissimilar flower types (W2, Y2; W2, R2; W3, P1; W3, P3; F1, P3). One would expect that the flower types with similar morphology or color would attract the same species of pollinators in the same rank order. For instance, if potential pollinator number 2 was the third most abundant species on flower type W-2, it should be the third most abundant species on W-3 if the morphology and color of the plants influence visitation to the flower types. This test

TABLE VIII

SPEARMAN RANK CORRELATION OF THE RELATIVE ABUNDANCE OF THE POLLINATORS AS THEY VISIT THE FLOWER TYPES*

| Flower Type | Flower Type | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | W-2 | W-3 | F-1 | F-2 | Y-2 | P-1 | P-2/4/6 | P-3 | P-5 | R-1 | R-2 | R-3 |
| W-2 | - | | | | | | | | | | | |
| W-3 | o | - | | | | | | | | | | |
| F-1 | o | o | - | | | | | | | | | |
| F-2 | o | o | X | - | | | | | | | | |
| Y-2 | X | o | o | o | - | | | | | | | |
| P-1 | o | X | o | o | o | - | | | | | | |
| P-2/4/6 | o | o | o | o | o | o | - | | | | | |
| P-3 | o | X | X | o | o | X | o | - | | | | |
| P-5 | o | o | o | o | o | o | o | o | - | | | |
| R-1 | o | o | o | o | o | o | o | o | o | - | | |
| R-2 | X | o | o | o | o | o | o | o | o | o | - | |
| R-3 | o | o | o | o | o | o | o | o | o | o | o | - |

*X's denote a significant correlation (P=.05).

indicates that the morphology and color of the flower types are not important determinants of pollinator visitation to those flower types.

In the next analysis I also used the Spearman rank correlation. I tested for correlations, in a pair by pair manner, the rank order of the flower visiting preferences of the bumblebee and silver-spotted skipper individuals that foraged for nectar (Table IX). If the pollinators' tongue lengths and the corolla tube length affect the flower type preference of the pollinators, then the rank order of the flower type preferences of the long-tongued pollinators should not be correlated with that of the short-tongued pollinators. A long-tongued potential pollinator may heavily visit the flower types with long corollas but one would not expect the short-tongued bumblebees to visit these flower types because they may be excluded from the nectar resource.

The flower visiting preferences of the two long-tongued bumblebees, B. vagans and B. bimaculatus, and one short-tongued bumblebee, B. affinis, are correlated. The flower visiting preferences of B. affinis are also correlated with the flower visiting preferences of B. impatiens (medium-tongued) and the silver-spotted skipper. The tongue length of the bumblebees does not seem to influence their flower visiting preference.

TABLE IX

SPEARMAN RANK CORRELATION OF THE FLOWER VISITING PREFERENCES OF THE POLLINATORS*

| Insect | B. perplexus | B. vagans | B. affinis | B. bimaculatus | B. impatiens | Silver-spotted skipper |
|---|---|---|---|---|---|---|
| B. perplexus | - | | | | | |
| B. vagans | o | - | | | | |
| B. affinis | o | X | - | | | |
| B. bimaculatus | o | X | X | - | | |
| B. impatiens | X | X | X | o | - | |
| Silver-spotted skipper | o | o | X | o | o | - |

*X denotes a significant correlation (P=.05).

Discussion

There is a distinct difference in the attractiveness of the flower types to the potential pollinators. The rare flower types are highly attractive to the pollinators in comparison to the more common red flower types. Morphological characteristics of the pollinators and flower types do not affect the flower type preference of the pollinators (Tables VII, VIII, and IX). Long-tongued bumblebees and butterflies did not exclusively visit the flower types with long corollas, nor did the short-tongued bumblebees exclusively visit the flowers with short corollas.

The short-tongued bumblebees are often excluded from the nectar resource of flowers that have long corolla tubes. These bumblebees often resort to nectar-robbing (a process whereby a flower visitor bites a hole in the corolla tube and takes the nectar without effecting pollination) to acquire nectar from flowers with long corollas. Very little nectar robbing was observed among the Gregory Bald azaleas in contrast to the closely related pinxter-flower in which almost every flower in two populations observed had been nectar-robbed. The reason that nectar-robbing is not more prevalent on Gregory Bald may be that the tougher corolla of many of the flower types is more difficult to bite through than the corolla of pinxter-flower. The short-tongued bumblebees may not have been excluded from the nectar resource on Gregory Bald, and it may not be energetically

profitable to rob nectar if nectar is available without expending unnecessary time and energy.

One reason that related flower types are not correlated is because the bumblebee species and some of the flower types are locally abundant on different areas of the bald. Some of the flower types tend to aggregate in clumped dispersion patterns and the bumblebees may be more numerous near their nest than far from their nest. A particular flower type may receive many visits from a certain bumblebee species because both are abundant in the same area.

Although there are differences in the morphology of the flower types (corolla tube length) and the pollinators (tongue length), these differences do not seem to affect the flower type preference of the pollinators. The next section discusses the behavior of the bumblebees while foraging for nectar or pollen. The pollen or nectar preference of a bumblebee may affect its flower type preference.

## VII. THE BEHAVIOR OF BUMBLEBEES FORAGING FOR NECTAR OR POLLEN

### Methods

The bumblebees foraged for both pollen and nectar while visiting the azalea flowers. I examined the pollen or nectar foraging preference of the species of Bombus and Andrena as they visited azalea plants in selected plots.

I also examined the attraction modes of the azaleas (pollen or nectar) to determine if one was more important than another for attracting bumblebees. For analysis, these data were arranged as the total number of visits to a flower type for pollen or nectar by a bumblebee species. The data were analyzed by flower type (to determine whether pollen or nectar was the more important attractant) and by bee species (to determine if the bumblebees or Andrena showed a preference for pollen or nectar). Wilcoxon's two-sample test (Sokal and Rohlf 1969) was used to analyze the data at P=.05.

A time record was also kept of pollinator activity and I made comparisons of the number of seconds that the bumblebees spent on a plant collecting pollen or nectar. I also used Wilcoxon's two-sample test to analyze those data (P=.05).

Results and Discussion: Attractiveness of Pollen or Nectar

Although the bumblebees collected both pollen and nectar from the azaleas, they were usually constant to either pollen or nectar during the same foraging trip. The number of bumblebee visits for pollen is not significantly different from those for nectar within a flower type (Wilcoxon's two-sample test, P=.05). The flower types do not differ in this respect. Table X summarizes the data. The difference in the attractiveness of the flower types is

TABLE X

THE NUMBER OF VISITS FOR POLLEN OR NECTAR TO THE FLOWER TYPES BY THE BUMBLEBEE SPECIES*

| Flower Type | Number of Visits For Pollen | Nectar |
|---|---|---|
| W-3 | 25 | 20 |
| W-2 | 10 | 20 |
| Y-2 | 4 | 4 |
| F-1 | 6 | 14 |
| F-2 | 39 | 34 |
| P-1 | 40 | 51 |
| P-2/4/6 | 7 | 5 |
| P-3 | 26 | 13 |
| P-5 | 18 | 13 |
| R-1 | 19 | 34 |
| R-2 | 26 | 48 |
| R-3 | 73 | 97 |
| Total | 293 | 353 |

*Both unidentified and identified bumblebee species were used in this analysis.

not related to a difference in the attractiveness of pollen or nectar to the pollinators.

Some of the pollinator species, however, do vary in their foraging; B. vagans and Andrena forage for nectar more often than for pollen and B. terricola and B. affinis forage more frequently for pollen (Wilcoxon's two-sample test, P=.05). Table XI summarizes these data. Bombus affinis and B. terricola are both short-tongued bumblebees, and they may be excluded from the nectar resource because their tongues are not long enough. These two species do not, however, forage for nectar more often on the flower types with the shorter corollas. The tongue length may affect foraging preference for pollen or nectar but it does not affect flower type preference. The pollen or nectar foraging preference of a bumblebee species may also be a function of the timing of the reproductive cycle. Bumblebees that are members of a nest that contains many larvae may forage for pollen more often than nectar.

Results and Discussion: Difference in the Amount of Time Spent Foraging for Pollen or Nectar

To determine if there is a difference in the amount of time the bumblebees spend foraging for nectar and pollen (intra- and interspecific differences), Wilcoxon's two-sample test was used to compare the pollen and nectar foraging behavior of the bumblebee species (Tables XII and XIII).

TABLE XI

POLLINATOR FORAGING PREFERENCE

| Pollinator | Number of Visits For Pollen | Nectar |
|---|---|---|
| B. perplexus | 61 | 113 |
| B. vagans* | 14 | 89 |
| B. affinis* | 50 | 15 |
| B. bimaculatus | 48 | 59 |
| B. impatiens | 92 | 59 |
| B. terricola* | 13 | 4 |
| Andrena* | 5 | 36 |

*Denotes a statistical difference in the number of foragers for nectar or pollen (Wilcoxon's Two-Sample Test, P=.05).

TABLE XII

A COMPARISON OF THE TIME SPENT BY EACH SPECIES OF BUMBLEBEE FORAGING FOR POLLEN, NECTAR OR BOTH POLLEN AND NECTAR

| | Species | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Bombus impatiens | | | Bombus perplexus | | | Bombus bimaculatus | | | Bombus vagans | | | Bombus affinis | | | |
| | B[a] | P | N | N | B | P | N | B | P | N | P | B | N | P | B | |
| Number observed | (12) | (36) | (72) | (87) | (4) | (38) | (51) | (11) | (52) | (53) | (9) | (0) | (13) | (51) | (4) | |
| Time[b] | 86.4 | 61.6 | 58.2 | 80.0 | 69.5 | 29.4 | 77.4 | 64.5 | 35.7 | 94.7 | 12.1 | 00 | 89.5 | 62.6 | 60.3 | |

[c]

[a]N = nectar forager, P = pollen forager, B = nectar and pollen forager.

[b]Time (in seconds) is the average value of the length of time each species spent foraging on a plant.

[c]Any pair of means underlined by a single line is not significantly different (Wilcoxon's two sample test, P=.05).

TABLE XIII

INTERSPECIFIC COMPARISON OF THE AVERAGE TIME BUMBLEBEES SPENT COLLECTING POLLEN, NECTAR OR POLLEN AND NECTAR[a]

| | Pollen | | | | | | Nectar | | | | | Pollen and Nectar | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Species | Ba | Bi | Bt | Bbm | Bv | Bp | Bv | Ba | Bp | Bbm | Bi | Bi | Bp | Bbm | Ba |
| Number observed | (51) | (36) | (8) | (52) | (9) | (38) | (53) | (13) | (87) | (51) | (72) | (12) | (4) | (11) | (4) |
| Time[a] | 62.6 | 61.6 | 38.8 | 35.8 | 12.1 | 29.4 | 94.7 | 89.5 | 80.0 | 77.4 | 58.2 | 86.4 | 69.5 | 64.5 | 60.3 |

[a]Time (in seconds) is the average value of the length of time each species spent foraging on a plant.

[b]Any pair of means underlined by a single line is not significantly different (Wilcoxon's two sample test, P=.05).

Bombus vagans and B. bimaculatus, both long-tongued, B. perplexus and B. affinis, a medium-tongued and a short-tongued, respectively, all spent a longer time collecting nectar than pollen on a plant. It seems unlikely that there is a relationship between the length of a bumblebee's tongue and the length of time it spends foraging for nectar or pollen on a particular plant. It further seems unlikely that a pollinator's foraging preference (pollen or nectar) affects the length of time that a pollinator spends collecting pollen or nectar. Bombus vagans and B. perplexus both spent more time collecting nectar than pollen. B. vagans is a more frequent collector of nectar than pollen while B. perplexus is not. Bombus affinis collects pollen more often than nectar yet she will spend a longer time collecting nectar than pollen on an individual plant.

These time differences may be an adaptation to reduce agonistic competition between the species of bumblebees. The less time an individual bumblebee spends on a plant, the less chance that an encounter will occur with an individual of another species. Agonistic encounters were observed between these bumblebee species, but they were rare occurrences.

In the next section I discuss the effect of time and temperature on pollinator activity.

# VIII. THE EFFECT OF TIME AND TEMPERATURE ON POLLINATOR ACTIVITY

## Methods

Certain pollinators are only active during specified times. This applies to hawkmoths (Gregory 1963, Hodges 1971) and bees (Linsley 1958, Linsley and Cazier 1963, Linsley, MacSwain and Raven, 1963, 1964). At the beginning of each plot sampling period, I recorded the time and temperature. I used regression analysis and chi square analysis to determine if the number of insect visitors to a plot varied with time of day or temperature. Regression analysis and chi square analysis were used to analyze the bumblebee data but only chi square analysis was used on the other insect visitors because of a smaller sample size. The data from all the plots were summed together to get a large sample size. Two regression lines were calculated for the bumblebees: a morning regression, 8:00-14:00 hours; and an afternoon regression 14:01-21:00 hours.

The mathematical model for the regression lines is specified by the following equation:

$$Y = B_1 \text{ Time} + B_2 \text{ Temperature} + A + E \text{ where}$$

E is a random normal variable (not shown in my equations) and A is the Y-intercept which determines the position of the regression line. $B_1$ measures the expected change in Y for a change in Time, Temperature remaining constant.

$B_2$ measures a change in Y for any change in Temperature, Time remaining constant. Separate chi square analyses were done for temperature and time. The pollinators were divided into bumblebees, small bees (Andrenidae and Halictidae), the silver-spotted skipper and Vespula species.

Results

The F tests for the morning regression (equation 1) and the afternoon regression (equation 2) of the bumblebees were not significant at the 5% level.

1) Y = 0.16 Time + -0.63 Temperature + 11.68.

2) Y = -1.92 Time + -0.02 Temperature + 42.38.

The chi square analysis is presented in Tables XIV and XV. The raw data for these analyses are presented in Appendix II, Tables XXIX and XXX. The bumblebees were significantly more abundant in the early morning and late afternoon and at lower temperatures than expected, but they visited the plots less than expected during midday and at the higher temperatures. The silver-spotted skipper, Vespula spp. and the small bees foraged during the warmer temperatures and during the middle of the day.

Discussion

Bumblebees are able to regulate their thoracic temperature (Heinrich 1972, Heinrich and Raven 1972), but the other insect visitors are solely ectothermic. Bumblebees

TABLE XIV

CHI SQUARE ANALYSIS OF THE EFFECT OF TIME OF DAY ON POLLINATOR ACTIVITY*

| Insect | Time of Day | | | | | |
|---|---|---|---|---|---|---|
| | 8/9 | 10/11 | 12/1 | 2/3 | 4/5 | 6/7 |
| Bumblebees | 6.3 (+) | 0.8 | 3.4 (-) | 2.5 (-) | 1.0 (+) | 6.9 (+) |
| Silver-spotted skipper | 4.1 (-) | 0.2 | 1.5 (+) | 4.2 (+) | 0.0 | 6.2 (-) |
| Small Bees** | 2.9 (-) | 0.2 | 9.1 (+) | 0.4 (+) | 1.8 (+) | 5.8 (-) |
| Vespula spp. | 11.1 (-) | 4.9 (-) | 1.0 (+) | 9.7 (+) | 2.0 (+) | 7.0 (-) |

*The entries in the table are the chi square values; + indicate a significant deviation greater than expected; - indicates a significant deviation less than expected; blanks indicate no deviation from expected. $X^2 = 93$; significant at P=.05, 15 degrees of freedom.

**Andrenidae and Halictidae.

TABLE XV

CHI SQUARE ANALYSIS OF THE EFFECT OF TEMPERATURE ON POLLINATOR ACTIVITY*

| Insect | Temperature (° C) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 14.4-18.3 | 19 | 20.3 | 21.2 | 22.5 | 23.6 | 24.4-27.8 |
| Bumblebees | 12.4 (+) | 2.7 (+) | 1.1 | 0.0 | 3.3 (-) | 4.5 (-) | 4.6 (-) |
| Silver-spotted skipper | 10.2 (-) | 0.9 | 1.6 | 0.1 | 2.6 (+) | 2.9 (+) | 3.6 (+) |
| Small Bees | 7.4 (-) | 3.0 (-) | 0.0 | 5.2 (+) | 0.3 | 1.9 | 0.2 |
| Vespula spp. | 16.9 (-) | 3.4 (-) | 3.3 (-) | 4.4 (-) | 8.7 (+) | 8.5 (+) | 14.2 (+) |

*The notation is the same as in Table XII, page 45. $X^2$ = 128; significant at P=.05, 18 degrees of freedom.

are also able to forage at lower temperatures than the other insects because they are not solely dependent on ambient temperature to become warm enough to be active. Heinrich (1972) observed bumblebees foraging at a temperature of 4° C. On Gregory Bald the bumblebees began their activity at 6:00 hours and foraged until 21:30 hours on nights that were light. The other flower visitors were not active as early as the bumblebees, and they disappeared sooner in the afternoon. Vespula species were less active at lower temperatures than the small bees and the silver-spotted skipper. In light of the physiology of the flower visitors, it is logical that the regressions are not significant for the bumblebees and that the chi square analyses indicate that the other pollinators are more active at higher temperatures and during the middle of the day.

A regression of other environmental variables besides time and temperature on pollinator activity might provide a better description of pollinator activity. Light intensity, presence or absence of sunshine, wind speed, degree of rain or mist in the air and relative humidity are other important environmental variables that affect pollinator activity. Although bumblebees produce their own thoracic heat, their activity is affected by the above environmental variables and it would be interesting to see if regression accurately predicts their activity. A bias involved in this analysis is the changeability of the

weather within the 20-minute plot sampling period. Nevertheless, any decrease in the amount of time spent observing the plot would result in a smaller number of insect visitors to the plots and probably a nonsignificant test.

The bumblebees visit the flowers over a wide range of temperatures and times of day but the other pollinators restrict their activity to the higher temperatures and midday. Certain plant species release pollen and nectar at specific times of day or temperatures, presumably in response to selection by pollinators. The next section examines the effect of time and temperature on pollen and nectar availability.

## IX. THE EFFECT OF TIME AND TEMPERATURE ON POLLEN AND NECTAR AVAILABILITY

Some plants are limited by time in the release of pollen (Percival 1955, Gregory 1963) and nectar (Baker 1961, Grant and Grant, 1965) and, in some, the availability of pollen and nectar is limited by temperature (Free 1968, Penfield et al. 1976 and Percival 1965). Such a mechanism would severely affect the visitation pattern of the azaleas and could influence competition among the hybrids.

### Methods and Results

I checked the availability of pollen and nectar from the hybrids (N=7) at various times and temperatures. Pollen

and nectar were available at all times of day and night at which I checked. Nectar was produced at night. Pollen and nectar were also available throughout the range of temperatures experienced on Gregory Bald (15°-30° C). At night the plants are visited by moths, and it is to the plant's advantage to present its pollen and to produce its nectar at night in order to sustain nighttime pollination. There is no effect of temperature or time on the availability of nectar or pollen.

None of the aspects of floral biology and pollinator behavior satisfactorily explains the attraction of the pollinators to certain flower types. In the next section I examine the relationship between particular nectar characteristics and the attractiveness of these plants.

## X. NECTAR CHARACTERISTICS

In an effort to determine why certain flower types are more attractive than others, I studied the following nectar characteristics: the nectar amount (rate of nectar production during 24-hour periods), the rate of nectar production for 2-5 hour periods, the rate of nectar production for flowers with receptive stigmas versus flowers with stigmas past receptivity, the sugar concentration of the nectar, the presence of amino acids in the nectar and the types of constituent sugars present in the nectar.

The nectar amino acids and nectar constituent sugars will be discussed in subsequent subsections.

## Nectar Rate and Nectar Sugar Concentration

Methods. To measure nectar amount I inserted a one microliter Microcap® micropipet into the top nectar guide of the flower. The nectaries are located at the base of the ovary at the end of the corolla tube and they are diagrammed in Knuth (1909). Sometimes capillary action was sufficient to extract the nectar but on other occasions suction was necessary to extract a sample. I used an Epic pocket refractometer, calibrated to the refractive index of sucrose, to determine the percent by weight of the sugar present in the nectar. Nylon stocking pieces were used to cover the flowers to prevent the flower visitors from extracting the nectar before I could measure it.

The molecular weight of sucrose is twice that of fructose or glucose and the refractive index of equal concentrations of sucrose is twice that of glucose or fructose. There is no error involved in the determination of total nectar sugar concentration by percent weight because the refractive index and molecular weight of sucrose are twice that for glucose or fructose.

Results. I used a nested analysis of variance to analyze the results in this section unless otherwise

specified. There is a significant amount of variability associated with the amount of nectar produced among the flower types and days within plants (Table XVI). This is important because it indicates that different flower types secrete different amounts of nectar. The Student-Newman-Keuls multiple range test for unequal sample size (Sokal and Rohlf 1969) was used to determine which flower types secrete the most nectar (Table XVII). Flower type W-1 secretes more nectar than many of the other flower types. Flower type F-1 also secretes more nectar than many of the other flower types although the difference is not statistically significant (P=.05). Flower type W-3 (closely related to W-1, Appendix I) and flower type F-1 were both attractive plants to the pollinators (Table IV, page 29), perhaps because of a large nectar volume.

It is difficult to explain the difference among days in the amount of nectar secreted. Different flowers were used on different days as the flowers aged and were replaced. Nectar production may vary with the age of the flower or different flowers on the same plant may produce different amounts of nectar. The weather, especially temperature, may have affected the rate of nectar production. The amount of nectar collected during warm periods may be greater than that collected during cold periods. Many plants also peak in nectar production during certain times of the day (Faegri and van der Pijl 1971). I was unable to determine

TABLE XVI

NESTED ANALYSIS OF VARIANCE: AMOUNT OF NECTAR

| Source of Variation | Sum of Squares | df | Mean Squares | F Test* | Adjusted MS** | F Test* | Significance P=.05 |
|---|---|---|---|---|---|---|---|
| Flower types | 190 | 6 | 32 | | | 5 | X |
| Plants within flower types | 81 | 13 | 6.2 | | 6.3 | 1.0 | |
| Days within plants | 472 | 70 | 6.7 | 2 | 6.8 | | X |
| Within days | 682 | 237 | 3 | | | | |

*See Sokal and Rohlf (1969) for the calculation of the F tests.

**Mean squares were adjusted to compensate for unequal sample size.

TABLE XVII

MEAN SEPARATION: AMOUNT OF NECTAR IN FLOWER TYPES*

| | Flower Type | | | | | | |
|---|---|---|---|---|---|---|---|
| Flower Type | W-1 | F-2 | F-1 | R-3 | P-1 | R-1 | P-2 |
| Mean nectar volume (µl) | 3.73 | 3.07 | 2.79 | 1.98 | 1.65 | 1.64 | 1.62 |

*Means underlined with the same continuous line are not significantly different at the 5% level.

if such a periodicity of nectar flow exists in these Rhododendron hybrids. All of these factors may have contributed to the variability in my results.

The flower types did not differ with respect to the rate of nectar produced during time periods two to five hours long (Table XVIII). This is important because a perceptible difference in the amount of nectar secreted in short time spans might influence pollinator visitation. Most of the flowers not enclosed by nylon stockings contained no nectar. A rapid replacement of depleted nectar supplies may be adaptive for attracting large numbers of pollinators.

There were day to day differences in single plants just as there was with nectar production during 24 hour periods. The same factors are probably responsible for these differences in both cases.

I analyzed the rate of nectar production (N=72) and nectar amount (N=28) for flowers that had receptive stigmas and stigmas past receptivity. There was no difference (P=.05) in the nectar amount (nested analysis of variance) and in the rate of nectar production (one way analysis of variance) for flowers with receptive stigmas and stigmas past receptivity.

One would think that the flowers with stigmas past receptivity would produce less nectar than flowers with receptive stigmas. Nevertheless, the emphasis of the

TABLE XVIII

NESTED ANALYSIS OF VARIANCE: RATE OF NECTAR PRODUCTION

| Source of Variation | Sum of Squares | df | Mean Squares | F Test* | Adjusted MS** | F Test* | Significance P=.05 |
|---|---|---|---|---|---|---|---|
| Flower types | 1.4 | 6 | .23 | | | 3.15 | |
| Plants within flower types | .27 | 7 | .04 | | .07 | .12 | |
| Days within plants | 5.97 | 18 | .33 | 3.79 | .32 | | X |
| Within days | 6.65 | 76 | .09 | | | | |

*See Sokal and Rohlf (1969) for the calculation of the F tests.

**Mean squares were adjusted to compensate for unequal sample size.

selection pressure exerted by the pollinators seems to be on the production of many blossoms that contain nectar. Within a particular flower type plants with few flowers attracted few pollinators compared with the plants that produced many flowers. Manning (1956) showed that bumblebees can be attracted to the forms of plants from a distance. An azalea plant with many flowers certainly stands out more than an azalea with few flowers. Once attracted to a plant, some of the pollinators are bound to visit flowers with receptive stigmas. This feature of the bloom is especially important in attracting pollinators to the plants when there are only a few plants on the bald and there are few pollinators visiting them.

The nectar sugar concentrations do not vary among the flower types but they do vary among the plants within a flower type (Table XIX). The means of the sugar concentrations ranged from 10.8% to 30.0% among the plants. These two extreme means are the only ones that are significantly different from each other (P=.05, Student-Newman-Keuls multiple range test) and both came from plants that are almost identical. Nectar production tends to be highly variable and the percent sugar in the nectar ranged from 7% to 70% in these azaleas. Rain caught in the corollas had little effect on the nectar sugar concentration because hairs on the filaments and style prevented contamination of the nectar by water (Leppik 1974 and personal observations).

TABLE XIX

NESTED ANALYSIS OF VARIANCE: PERCENT NECTAR SUGAR

| Source of Variation | Sum of Squares | df | Mean Squares | F Test* | Adjusted MS** | F Test* | Significance P=.05 |
|---|---|---|---|---|---|---|---|
| Flower types | 826.5 | 6 | 137.8 | | | 1.0 | |
| Plants within flower types | 2760.7 | 13 | 212.4 | | 135.4 | 3.6 | X |
| Days within plants | 1530.2 | 24 | 63.8 | .7 | 59.3 | | |
| Within days | 6992.9 | 73 | 95.8 | | | | |

*See Sokal and Rohlf (1969) for the calculation of the F tests.

**Mean squares were calculated to compensate for unequal sample size.

Relative humidity affects the concentration of sugars in nectar because water is hygroscopic (Ortel 1944, Park 1929 and Shuel 1956), but changes in the relative humidity do not affect nectar secreted into a long and narrow corolla tube (Park 1929 and Percival 1965). There was a tendency for the nectar of flowers enclosed by nylon stockings to be less concentrated than the nectars of exposed flowers. This might be a result of humidity differences between enclosed and exposed flowers. Ortel (1946) reported that the sugar concentration in nectar extracted from alfalfa growing in conditions of constant humidity varied from 11% to 38%. Heinrich's (1976b), Ortel's (1944, 1946), and my data indicate that nectar volume and sugar concentration are highly variable within a species.

The amount of nectar sugar in the flowers was calculated by multiplying the percent nectar sugar times the nectar volume. The attractiveness of individual plants to bumblebees is correlated to the micrograms of nectar sugar produced by the plants (Spearman rank correlation, P=.05, Table XX). The attractiveness was calculated by dividing the number of visits by bumblebees to a plant by the number of times the plant was observed for visitors. I also tested for a correlation between the attractiveness of the flower types and the micrograms of sugar a flower type produced. This test was not significant (P=.05), largely because the least attractive flower type (R-2/3) produced the most

TABLE XX

SPEARMAN RANK CORRELATION BETWEEN THE ATTRACTIVENESS TO BUMBLEBEES OF INDIVIDUAL PLANTS AND MICROGRAMS OF NECTAR SUGAR*

| Flower Type | Plant | Rank of Attractiveness to Bumblebees | Micrograms of Nectar Sugar |
|---|---|---|---|
| F-2 | XIV-1 | 1 | 1.15 |
| P-1 | XII-1 | 2 | 0.79 |
| P-1 | XIII-3 | 3 | 0.53 |
| F-1 | XIII-1 | 4 | 0.71 |
| P-2 | XIII-2 | 5 | 1.63 |
| R-1 | XIV-4 | 6 | 0.41 |
| F-2 | XII-3 | 7 | 0.40 |
| R-3 | XII-x | 8 | 1.03 |
| P-2 | XII-2 | 9 | 0.30 |
| P-2 | XII-7 | 10 | 0.20 |
| F-2 | XII-6 | 11 | 0.49 |

*The correlation is significant; $r_S$ = .627, P=.05.

sugar and because of the small sample size (Table XXI). Although this result is not statistically significant, there is a tendency for the pollinators to forage on plants that are the richest energetically. This is an important finding and corroborates the work of other pollination ecologists (Faegri and van der Pijl 1971, Proctor and Yeo 1972).

Nectar Amino Acids

Baker and Baker (1973) and Watt et al. (1974) discussed the importance of nectar amino acids for butterfly pollinators. The floral morphology of Rhododendron spp. suggested to Percival (1965) that they are pollinated by bumblebees or butterflies. Since the flower morphology of the hybrid azaleas of this study varied widely, I reasoned that butterflies might be effective pollinators of certain flower types.

Methods. I spotted the nectar samples onto a piece of Whatman chromatography paper in the field. In the laboratory I sprayed them with ninhydrin and dried them for ten minutes in an oven at 100° C. I checked 17 different plants and six different flower types (W1, F2, P1, P2, R1, R2) for the presence or absence of amino acids.

Results. Amino acids were found in all plants examined and butterflies did not seem to be important

TABLE XXI

SPEARMAN RANK CORRELATION BETWEEN THE ATTRACTIVENESS TO BUMBLEBEES OF FLOWER TYPES AND MICROGRAMS OF NECTAR SUGAR*

| Flower Type | Rank of Attractiveness to Bumblebees | Micrograms of Nectar Sugar |
|---|---|---|
| F-1 | 1 | .71 |
| P-1 | 2 | .79 |
| W** | 3 | .76 |
| F-2 | 4 | .68 |
| P-2/4/6 | 5 | .61 |
| R-1 | 6 | .38 |
| R-2/3 | 7 | 1.19 |

*The correlation is not significant; $r_s$ = .14, P=.05.

**Attractiveness values were calculated for W-2 and W-3, but nectar was collected only from W-1, a related flower type.

pollinators of any one flower type. There were some flower types that were attractive to the silver-spotted skipper (Hesperiidae), but these plants were also attractive to the bumblebees (Table VI, page 33). There does not seem to be any particular correlation between the presence of amino acids in a flower type and butterfly visitation to that flower type.

## Nectar Constituent Sugars

Methods. Wykes (1952) discovered in laboratory studies that honeybees preferred solutions containing equal parts of sucrose, glucose and fructose over solutions containing other combinations of sugars or solutions containing single sugars. I analyzed nectar samples ranging in quantity from 2 microliters to 7 microliters from 15 plants and seven flower types (W1, P1, P2, F1, F2, R1, R3) for their constituent sugars.

A Perkin Elmer 3920 gas chromatograph was used to detect the presence of the nectar sugars. The column was 6 feet long, one-quarter inch outside diameter and packed with chromosorb W, 3% SE 52. The nectar samples were collected in the field and put into one-half dram vials and saved for later analysis. The samples had to be dry before preparation for analysis. To prepare the samples for analysis 0.5 ml of silylating agent was added directly to the vial. After 5-10 minutes the sample was evaporated to

dryness and 1 ml of hexane was added to the vial. The hexane-silylated sugar mixture was then filtered and 1 ml of hexane was added to the filtrate and the mixture was again filtered. The mixture was evaporated to dryness and one or two drops of hexane were added as a solvent before injection into the gas chromatograph. The oven temperature of the machine was set at 180° C and the carrier gas (helium) flow rate was set at 50 ml per minute.

Results and Discussion. Sucrose and fructose were the only sugars present in the nectars of the hybrids. Percival (1961) examined the constituent sugars in 889 species of plants and discovered that flowers with tubular corollas tended to have nectars dominated by sucrose. She also examined the nectar constituent sugars in a number of unrelated Rhododendron species and found much variation. These sugar mixtures were pure sucrose, pure glucose and fructose, or various combinations and amounts of sucrose, glucose, and fructose. She found no correlation between the nectar constituent sugars and the attractiveness of the plants to honeybees among the plants she examined. Probably nectar amount and nectar sugar concentration are more important factors in the attraction of plants to bumblebees under field conditions than the presence of certain constituent sugars (Faegri and van der Pijl 1971).

Discussion of Nectar Characteristics

Amino acids were present in the nectar of all the flower types examined and the gas chromatogram pattern of the constituent nectar sugars does not differ among the flower types. Since these two nectar characteristics do not vary among the flower types, it is unlikely that they account for the difference in the attractiveness of the flower types. Amino acids in floral nectar may be helpful for gametogenesis in some butterflies, but butterflies were not important flower visitors.

There was significant variability in the nectar volume and percent nectar sugar. The plants that contained the greatest amount of sugar in the nectar received the most flower visits. This result corroborates Heinrich's (1976a) findings. The nectar volume and percent nectar sugar were the single most important factors in determining pollinator (specifically bumblebee) visitation.

# CHAPTER III

## THE CONSTANCY OF THE POLLINATORS TO THE FLOWER TYPES

The flower constancy of pollinators is emphasized in many papers (Heinrich 1975, Linsley 1958, Grant 1950, Free 1968, 1970). Nevertheless, if the data are examined closely, one notices that some species of bees are quite inconstant. Chambers (1945) examined the pollen loads of four species of Andrena and found that only one of the species was relatively flower constant. This constancy was determined by examining the species composition of the pollen loads of the bees. Free (1968) examined the pollen loads of honeybees and found that 20% of the bees were inconstant over a three-day period. The bees were more constant if examined on a daily basis. In a study of flower visits to Brassica napus L. and B. oleracea L., Bateman (1951) found that bumblebees were less flower constant than honeybees and that small bees were less flower constant than bumblebees. Oster and Heinrich (1976) discussed the adaptive value of a small amount of flower inconstancy to bumblebees.

Oster and Heinrich (1976) state:

> Unlike honeybees, bumblebees frequently sample flowers of several plant species at the beginning of their foraging career, but ultimately specialize on a particular species (hereinafter called their "majors") while continuing to occasionally visit alternate flowers (hereinafter called their "minors").

If the nectar rewards of the minors are greater than the majors, the bumblebees will switch from their majors to their minors (Heinrich 1976a). The process of minoring allows the bees to best exploit the resources available to them (Heinrich 1976a, Oster and Heinrich 1976).

## I. METHODS

The order in which potential pollinators visited flower types was recorded on selected of the plots previously described. Since few crosses between flower types occurred on the plots, the flower type categories were condensed in order to increase the expected values for chi square analyses (Table XXII). The expected values were calculated from the density of shrubs of various flower types within the plot. For instance, in a plot containing one white flowered, one purple flowered, and four red flowered shrubs, the expected values for a cross from the white flower type to the red flower type are: white, 0 (a plant cannot cross with itself); purple, 1/5; red, 4/5. Bateman (1951) used a similar chi square design to analyze the flower constancy of honeybees, bumblebees and small bees visiting plots containing Brassica napus and B. oleracea.

## II. RESULTS

The greatest variability in my results is in the P-R and R-P cells of Table XXII; there is a mutual avoidance

TABLE XXII

CHI SQUARE ANALYSIS OF THE CONSTANCY OF THE POLLINATORS TO THE FLOWER TYPES*

| | | Flower Type Visited Second | | | |
|---|---|---|---|---|---|
| | | W/F/Y | P | R | Value |
| Flower type | W/F/Y | 6 | 18 | 32 | Observed value (o) |
| | | 6.6 | 16.0 | 33.4 | Expected value (e) |
| visited | P | 24 | 33 | 6 | o |
| | | 19.7 | 23.9 | 20.2 | e |
| first | R | 35 | 2 | 140 | o |
| | | 28.1 | 7.5 | 140.3 | e |

*$X^2$ = 20.4; significant with four degrees of freedom.

of the red and purple flower types. This result may lead us to believe that the pollinators are flower type constant. Nevertheless, the pollinators are not flower type constant because the observed values are not much greater than the expected values for the WFY-WFY, P-P and R-R cells. If the flower visitors were flower type constant, a bee that visits a purple flower the first time will visit a purple flower the second time as well; therefore, the observed visits in the P-P cell would be greater than expected if the pollinators were flower constant.

Many of the cells indicate that the visitation pattern of the pollinators is random. Lumping the flower types together, however, decreases the sensitivity of the test. Flower type W-3 was involved in more crosses than expected (P=.05, chi square) because flower type W-3 was an attractive plant and it received many insect visits. The more attractive plants in general are probably involved in more crosses than the less attractive plants. The chi square is not significant for the other flower types because the flower visitors often went from plants within a plot to those outside the plot and that would not show up in the analysis.

## III. DISCUSSION

The most attractive flower types were also the rarer flower types. It is not energetically efficient to be

flower constant to a rare flower type because more energy is expended in the collection of nectar than is present in the amount of nectar collected. Flower constancy was observed, however, between two highly attractive plants growing in the same area. The bumblebees would probably be more constant to the flower types if the attractive flower types grew close together. Heinrich (1976a) discussed an experiment by Kugler (1955) that showed that bumblebees visited red and white flowers of Lathrys in a random fashion. When sugar water was added to the red flowers, the bees visited the red flowers more often than the white flowers. The morphology of the two Lathrys color morphs and the azalea hybrids does not differ significantly enough to cause a loss of efficiency to the bumblebee as she visits different color morphs of Lathrys or different azalea flower types. If the morphology of the Lathrys color morphs or of the azalea flower types differed, the bumblebees might exhibit more flower constancy.

Bateman (1951) also observed the crossing behavior of pollinators within plots. His plots differed from mine in that they contained equal numbers of Brassica napus and B. oleracea. Bumblebees were flower constant to B. oleracea while honey bees were constant to B. napus. It would be interesting to see the effect of varying densities of these plants on the flower constancy of these bees.

Minoring is essential to the bumblebees in order for them to discover the flower types that contain the most energy in the nectar. A certain amount of inconstancy is highly adaptive because the similar flower morphology of the azalea hybrids does not cause a decrease in foraging efficiency. Bumblebees are flower type inconstant in order to continuously monitor the nectar sugar amount in the various flower types, thus enabling them to forage more extensively on the flower types that produce the most nectar sugar. Heinrich (1976a) observed that bumblebees chose an alternate food source when their primary food source was eliminated. The bumblebees on Gregory Bald switched to alternate flower types as their major flower types completed blooming and other flower types began to bloom. This was especially noted at the end of the blooming season when the flower visiting pattern approached randomness. I was able to distinguish a few bumblebees from the rest of the bumblebees by their individual behavior patterns. Those bumblebees followed individual foraging routes among the few remaining flower types. Two main causes of the azalea hybridization are the flower type inconstancy of the bumblebees and the bumblebee's ability to shift from one flower type to another.

# CHAPTER IV

## SELF-POLLINATION AND APOMICTIC REPRODUCTION

To help determine the effect of the pollinators' flower visitation behavior on seed set, various aspects of the reproductive biology of the plants were studied. The species of azaleas that form the hybrid swarm on Gregory Bald all produce seed by self-pollination, apomixis or cross-pollination (Galle, personal communication). I performed experiments on the flower types to determine the extent of self-pollination and apomictic reproduction within the flower types. In one experiment I self-pollinated the flowers to determine the extent of self-compatibility among these azaleas. In another experiment I excluded the pollen vectors from the flowers to determine if the flowers can "passively" pollinate themselves. Seed set from control flowers was compared to that of the experimentally manipulated flowers to determine if differences existed between the control and experimental groups.

### I. METHODS AND RESULTS

The majority of the plants tested for self-compatibility did not produce seed (Table XXIII). One hundred and two flowers from 20 plants and nine flower types (W2, W3, F2, Y1, P3, P5, R1, R2, R3) produced no

TABLE XXIII

THE SEED SET OF THE HAND SELF-POLLINATED FLOWERS

| Flower Type | Plant Number | Number of Flowers Pollinated | Number of Capsules Produced | Number of Seeds in the Capsules |
|---|---|---|---|---|
| W-2 | VI-30 | 2 | 0 | 0 |
| W-2 | VI-16 | 5 | 0 | 0 |
| W-2 | IV-32 | 3 | 0 | 0 |
| W-3 | I-1 | 7 | 0 | 0 |
| W-3 | VII-17 | 6 | 1 | 0 |
| F-2 | VIII-4 | 6 | 1 | 28 |
| F-2 | IX-2 | 10 | 2 | 0 |
| F-2 | IV-34 | 5 | 0 | 0 |
| P-5 | VII-14 | 4 | 0 | 0 |
| P-5 | VII-7 | 4 | 0 | 0 |
| P-3 | VIII-3 | 2 | 0 | 0 |
| R-1 | VIII-1 | 7 | 0 | 0 |
| R-1 | I-8 | 5 | 0 | 0 |
| R-1 | IX-3 | 4 | 0 | 0 |
| R-3 | IV-5 | 5 | 0 | 0 |
| R-3 | VII-2 | 5 | 0 | 0 |
| R-3 | V-8 | 5 | 0 | 0 |
| R-2,R-3 | IX-1 | 5 | 2 | ? |
| R-2,R-3 | VII-18 | 5 | 0 | 0 |
| Y-1 | VII-17 | 6 | 0 | 0 |
| Total | | 101 | 6 | 28 |

apomictic seed. To test for apomixis I cut the anthers from the stamen while the flower was in bud. In some species pollination is necessary for apomixis to occur (pollination does not imply fertilization). If this is true for these azaleas, my test will not determine the importance of apomictic reproduction in the azalea hybrid swarm. Fred Galle (personal communication) cross-pollinated some of the hybrids and discovered that there was some cross incompatibility among the hybrids, indicating that apomictic reproduction may not be very important in this hybrid swarm.

Leppik (1974) states that if cross-pollination does not occur after a period of time in R. nudiflorum, the stigma moves near the anthers and insures self-pollination. To test for such a mechanism in the Gregory Bald azaleas, I designed flower enclosures that were made from one-half gallon milk cartons cut in half and covered with nylon stockings. These flower enclosures were designed to allow self-pollination without the help of flower visitors. Occasionally the reproductive parts of the flower would touch the side of the enclosure. This bias, however, is not very significant in light of the poor seed set (Table XXIV). Observations of the positions of the stigmas and anthers indicated that insect mediated self-pollination may happen occasionally, but in the majority of cases the

TABLE XXIV

THE SEED SET OF FLOWERS BAGGED TO EXCLUDE POLLEN VECTORS

| Flower Type | Plant Number | Number of Flowers Enclosed | Number of Capsules Formed | Number of Seeds in Each Capsule |
|---|---|---|---|---|
| W-2 | VI-30 | 6 | 0 | 0 |
| W-2 | VI-6 | 7 | 0 | 0 |
| W-2 | IV-32 | 5 | 0 | 0 |
| W-3 | I-1 | 5 | 1 | 14 |
| W-3 | I-13 | 6 | 6 | capsules eaten* |
| F-2 | IX-2 | 6 | 1 | capsules eaten* |
| P-5 | VII-14 | 6 | 0 | 0 |
| P-5 | VII-7 | 7 | 0 | 0 |
| P-3 | VIII-3 | 6 | 0 | 0 |
| R-1 | VIII-1 | 6 | 0 | 0 |
| R-1 | I-8 | 2 | 0 | 0 |
| R-1 | IX-3 | 4 | 0 | 0 |
| R-3 | IV-5 | 6 | 0 | 0 |
| R-3 | V-8 | 5 | 0 | 0 |
| R-2,R-3 | IX-1 | 6 | 1 | ? |
| R-2,R-3 | VII-19 | 5 | 1 | 18 |
| Y-2 | VII-17 | 11 | 0 | 0 |
| Total | | 99 | 10 | 32 |

*A coleopterous or lepidopterous larva devoured the contents of the capsule during seed development before the seeds were counted.

stigma is spatially separated from the anthers and selfing does not occur.

To determine if the seed set from unaltered flowers is any different from the seed set of the flowers involved in the pollination experiments, an umbel of two to seven old flowers or newly forming capsules was selected on each plant on which a pollination experiment was carried out. Seeds in the developing capsules were counted in late October. The capsule formation and seed set (Table XXV) are less than the reproductive capacity of the azaleas. Lee (1965) and Galle (1974) report the seed set of human cross-pollinated flowers as 200-500 seeds per capsule. The average seed set of the Gregory Bald azaleas was 29 seeds per capsule and only one capsule contained nearly 200 seeds. Since some flowers did not produce capsules, the average seed set per flower (4.25) was considerably less than the average number of seed per capsule.

## II. DISCUSSION

The pollination of these azaleas by flower visitors may not be very efficient. The low seed set of the controls on Gregory Bald and general observations of the seed set of flame azalea and pinxter-flower indicate that the number of capsules is small in comparison to the number of flowers produced. Observations on the flower visiting behavior of the pollinators support the hypothesis that the pollination

TABLE XXV

CONTROL SEED SET

| Flower Type | Number of Flowers Selected | Number of Capsules Formed | Number of Seeds in Each Capsule |
|---|---|---|---|
| W-2 | 2 | 0 | 0 |
| W-2 | 8 | 5 | 7,41,18,6,5 |
| W-2 | 5 | 0 | 0 |
| W-3 | 6 | 0 | 0 |
| W-3 | 4 | lost | --- |
| W-3 | 2 | 0 | 0 |
| W-3 | 4 | 1 | 2, capsule decaying |
| W-3 | ? | 0 | 0 |
| Y-1 | 5 | 2 | 12,71 |
| F-2 | 5 | 0 | 0 |
| F-2 | 7 | 0 | 0 |
| F-2 | 5 | 0 | 0 |
| P-1 | 19 | 3 | 17,12,39 |
| P-1 | 18 | 3 | 9,22,? |
| P-2 | 15 | 1 | ? |
| P-3 | 2 | 0 | 0 |
| P-5 | 10 | 3 | 16,21,22 |
| P-5 | 8 | 0 | 0 |
| R-1 | ? | 1 | 197 |
| R-1 | 6 | 2 | 24,24 |
| R-1 | 5 | 0 | 0 |
| R-1 | 5 | 0 | 0 |
| R-3 | 4 | 0 | 0 |
| R-3 | 6 | 0 | 0 |
| R-3 | 6 | 1 | 13 |
| R-3 | 5 | 0 | 0 |
| R-3 | 4 | 1 | ? |
| R-3 | 9 | 0 | 0 |
| R-2,R-3 | 4 | 0 | 0 |
| R-2,R-3 | 4 | 1 | 55 |
| R-2,R-3 | 5 | 1 | 9 |
| Total | 178 | 25 | 632 |

mechanism is inefficient. The pollinators do not touch the stigmas very often and when they do touch the stigma the chances of pollen transfer are remote. The Gregory Bald azaleas have further difficulties producing seed because some of the hybrids are cross-sterile (Galle, personal communication).

Drought is another factor that affects the seed set of azaleas (Galle, personal communication). There may have been a drought in July on Gregory Bald, but the rainfall for other months was at or above average (TVA 1977). The rainfall for July at Cades Cove (1716 feet and 7.5 mi. from Gregory Bald) was 3 inches below average but the rainfall at Newfound Gap (5300 feet and 25 mi. from Gregory Bald) was one inch above average. The rainfall in the Great Smoky Mountains is typically greater at the higher elevations than the lower elevations. In light of this information there probably was not a drought in July on Gregory Bald, but there is no way to know for certain.

# CHAPTER V

## SUMMARY

Baseline data were gathered on the pollination ecology of the azaleas on Gregory Bald in order to help understand the origin of the azalea hybrids growing there. These azaleas are adapted to outcrossing through protandry. The floral morphology of these azaleas indicates that they are primarily adapted to pollination by flower visitors, and that wind pollination is not very important in contributing to the reproduction of these plants. The initiation of nectar production, which coincides with the ripening of the stigmas, may be an indication that these plants are adapted to pollination by a nectar gathering flower visitor. Examination of the pollination efficiencies of the flower visitors indicated that the pollen and nectar gathering bumblebees are the most important pollinators. The pollen gatherers, however, are more efficient at pollination than the nectar foragers. Nectar foraging moths and hummingbirds are more efficient pollinators than the bumblebees but they are fewer in number than the bumblebees.

Certain flower types attract more flower visitors than other flower types. The following aspects of the bloom of the azaleas were studied in an attempt to explain this difference in attractiveness: the effect of temperature

and time on nectar and pollen availability, the presence of amino acids in the nectar, the constituent sugar content of the nectar, the nectar volume and the nectar sugar concentration.

The effect of time or temperature on the availability of pollen or nectar did not differ among different flower types. Nectar is also produced at night. Some of the azalea flower types have characteristics that typify moth pollinated flowers: light floral coloration, odor production, and a long slender corolla tube (Baker 1961). These factors indicate that moths may visit these flowers and indeed moths did pollinate flowers on a few of these hybrids.

Time of day and temperature did not affect bumblebee activity as it did the activity of the other diurnal flower visitors. Bumblebees are active at low temperatures because they are able to produce their own thoracic heat and are not dependent on ambient temperature for flight. Azalea plants have responded to pollinator selection pressure by presenting their pollen and nectar continuously with no time or temperature restriction.

The morphologies of the flower types (corolla tube length and diameter) and the bumblebee species (tongue length) vary significantly, but are not correlated. Flowers with long corolla tubes are visited by both long-tongued and short-tongued bumblebees and the flower types with

short corolla tubes are also visited by both long-tongued and short-tongued bumblebees. The pollinators also did not show a flower color preference, although they did not visit the red flowered plants as often as they did plants of other flower colors. The tongue length did affect the foraging preference of the bumblebees. The short-tongued bumblebees, B. affinis and B. terricola, did prefer to forage for pollen instead of nectar. One long-tongued bumblebee, B. vagans, preferred to forage for nectar, while the other long-tongued bumblebee, B. bimaculatus, showed no foraging preference.

The gas chromatogram pattern and the presence of nectar amino acids did not differ among the flower types. The presence of certain constituent sugars and amino acids did not seem to influence pollinator visitation to the flower types. The silver-spotted skipper showed a preference for certain flower types, but this was not correlated with the presence of amino acids in the nectar. The flower types that this butterfly preferred were also preferred by some bumblebees. These butterflies may prefer nectar that contains a large amount of sugar.

The pollinators tended to visit the flower types that contained the most sugar in their nectar. Heinrich's (1976a) work on the foraging activity of bumblebees indicates that nectar amount is an important variable in correlating pollinator-plant attraction. Bumblebees need

to realize an energy profit while foraging to maintain the fitness of the colony. Slight flower inconstancy allows the bumblebees to continuously monitor their nectar supply and to forage on those plants that contain the most nectar sugars. In this manner they discover which plants are the richest energetically, and they remain constant to those plants throughout the blooming period. In the event one flower type quits blooming, the bumblebees will visit others. Bumblebees maintain distinct foraging routes (Heinrich 1976a), and they forage on the same plant (flower type) on successive foraging trips. Once they discover an energetically rich plant, it remains in their foraging path until it finishes blooming. In this manner the attractive flower types are able to attract and maintain large numbers of faithful flower visitors.

The pollinator flight patterns were examined to determine the amount of flower type constancy exhibited by the pollinators. This has important implications for the direction of gene flow between the flower types. Although bumblebees in general exhibit flower constancy once their "majors" are chosen, they were not constant to flower types on Gregory Bald. Part of the reason for this may be that the energetically rich flower types were not very abundant on Gregory Bald and constancy to a rare flower type may not be a prudent foraging strategy. The pollinators must forage on both the rare and common flower types in order to maintain

an energetic profit. This type of foraging activity may promote more hybridization than found in a plant population in which the pollinators forage randomly. Had the rare flower types been more common on Gregory Bald, there might be fewer hybrids on the bald. At a higher density of the rare flower types the pollinators might remain constant to the rare flower types.

The foraging paths of the different pollinators were compared to determine if there were any differences in the foraging activities of the pollinators. The hummingbirds, butterflies and bumblebees were constant to flower types. The moths, however, were only observed on two adjacent clones of one flower type (P-1). During the summer of 1977 the moths were not active long enough to significantly affect the seed set of the azaleas. During some seasons the weather may be more conducive to moth activity and the moths may effect a significant amount of self-pollination or cross-pollination of a select group of flower types. The flower visitation behavior of the moths showed much more flower type constancy than did the foraging behavior of other pollinators. The type of seed set on Gregory Bald (whether from cross-pollination among different flower types or cross-pollination within a select few flower types) during any one season may depend on the abundance of any one pollinator type; nevertheless, bumblebees are the major pollinators of the azaleas and the

majority of the seed set on Gregory Bald during most seasons is probably from bumblebee activity.

The seed set of the azaleas on Gregory Bald is below the reproductive capacity of these plants. The reason for this is not known, but it may be that the fertilization and pollination efficiencies in these plants were low or it may be that drought affected the seed set. Preliminary observations of the seed set of other azalea species tend to support the hypothesis that the pollination and fertilization efficiencies are low. The seed set may fluctuate from year to year. It rained and was frequently foggy on Gregory Bald during the bloom of the azaleas in 1977 and this affected pollinator activity. During years in which the weather is more conducive to pollinator activity, more seed may be set. Another factor that may affect the seed set is the amount of pollen collected from the azaleas by the bees. In years in which the demand for pollen is not great, there may be more pollen available for pollination. Cross-sterility of some of the hybrids may also affect the seed set. The extent of this cross-sterility among the flower types is unknown and further work needs to be done in this area.

The small amount of seed set on Gregory Bald from the pollination experiments indicates that the azaleas are primarily outcrossing. This information is important if we are to discover if there is a greater tendency to

outcross (and hence hybridize) than to self-pollinate. Pollinator behavior that contributes to self-pollination of these azaleas may not significantly affect the reproduction of these plants.

# CHAPTER VI

## CONCLUSION

Energetic efficiency of bumblebees while foraging promotes the hybridization of the azaleas on Gregory Bald. The pollinators of the azaleas, specifically the bumblebees, were more attracted to certain flower types than others. The highly attractive flower types were the rarer ones and their nectar contained more sugar than the commoner flower types. In order for the pollinators to be energetically efficient while foraging, they cannot be flower constant to the more attractive plants (because of their rarity), but most also visit the less attractive plants.

Pollination experiments were undertaken to ascertain the effect of pollinator inconstancy on the seed set of the azaleas. A large amount of seed resulting from apomixis or self-pollination would negate the effects of the pollinators as hybridizers. Little self-pollination occurs among the azalea flower types but apomictic reproduction may occur. Cross-pollination is probably the major avenue to seed production in these azaleas since the hand pollination and apomixis experiments did not result in much seed production.

Galle's cross-pollination experiments indicated that in certain cases there are compatibility barriers between

certain hybrids. There is need for work to determine if there is a tendency for this incompatibility to exist between certain flower types. These incompatibility barriers may affect the seed set of certain flower types and may have an effect on the proliferation of certain flower types.

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## LITERATURE CITED


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# APPENDICES

# APPENDIX I

## FLOWER CHARACTERISTICS

### TABLE XXVI

### COLOR AND MORPHOLOGICAL CHARACTERISTICS OF THE FLOWER TYPES

| Flower Type | Corolla Tube Length (mm) | Corolla Diameter (mm) | Corolla Color** | Notes |
|---|---|---|---|---|
| White 1 | -- | -- | rp 62 D | These flowers are white except for slight coloration on the corolla edges. |
| White 2 | 16-18 (28)* | 27-35* | rp 62A,C; rp 67D | |
| White 3 | 19 (14) | 33-36 (29-32) | rp 62C; r55C/ y 13B;y 15A blotch | W-3 is similar to W-1 except that W-3 has more coloration. |
| Yellow 1 | -- | -- | yo 20C | |
| Yellow 2 | 14.5 (15-18) | 32-36 (36-44) | y 14C; yo 16A; yo 21C | |
| Flesh 1 | 17 | 35-40 | o 29A/ r 42D stripes | The flowers of F-1 have more orange coloration than the flowers of F-2. |
| Flesh 2 | 13-18 | 28-38 (25-29) | o 29B; yo 20B; yo 25A/ r 50B,C blotch sometimes | |
| Purple 1 | 17-20 | 27-36 | rp 62A; or 39B/ rp 58B,C blotch | |

TABLE XXVI (continued)

| Flower Type | Corolla Tube Length (mm) | Corolla Diameter (mm) | Corolla Color** | Notes |
|---|---|---|---|---|
| Purple 2 | 13-18 | 25-37 | r 47C; r48B/ r 50B blotches sometimes | |
| Purple 3 | 13-17 | 24-27;28-40 | r 55A; rp 57D | |
| Purple 4 | 13-16 | 30-37 | rp 63C,D | |
| Purple 5 | 12-16 | 30-37 | rp 58C | |
| Purple 6 | 14-15 | 34-37 | rp 57D | |
| Red 1 | 12-15;15-17 | 34-40;29-37 | r 42A; r 44A | R-1 is a deeper, darker red than the other red flower types. |
| Red 2 | 11-15;18-21 | 27-37;31-40 | or 32A,B,C | R-2 has more orange coloration than flower type R-3. |
| Red 3 | 13-18 | 30-40 (40-45) | r 41B; r 42B; r 44C | |

*Double entries indicate that there were two flower sizes, and entries within parentheses indicate a less common flower size.

**Color measurements were made with a Royal Horticultural Society Colour Chart (Anonymous 1966): r = red, p = purple, y = yellow, o = orange.

Multiple entries indicate a slight variation in the shades of a flower color among the flower types. (A single entry indicates that only one or a few plants were sampled. A slash separates entries for corolla color from those for blotch or strip color.) The comma separates similar colors of the same number while a semicolon separates similar colors of different numbers.

# APPENDIX II

## G-TESTS AND CHI SQUARE ANALYSES

### TABLE XXVII

### G-TEST OF THE ATTRACTIVENESS OF THE FLOWER TYPES TO THE BUMBLEBEES*

| Species | Flower Type W-2 | W-3 | F-1 | F-2 | Y-2 | P-1 | P-2/ 4/6 | P-3 | P-5 | R-1 | R-2 | R-3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B. perplexus | 21 | 13 | 5 | 11 | 2 | 12 | 14 | 19 | 18 | 21 | 39 | 57** |
| | 10.32 | 2.9 | 6.7 | 17.9 | 8.0 | 9.3 | 16.8 | 12.7 | 9.5 | 16.0 | 58.8 | 62.1++ |
| B. vagans | 8 | 9 | 12 | 5 | 5 | 5 | 1 | 4 | 3 | 15 | 20 | 44 |
| | 4.8 | 3.3 | 5.0 | 9.5 | 6.8 | 6.0 | 8.0 | 2.4 | 1.8 | 9.8 | 29.0 | 43.8 |
| B. affinis | 4 | 3 | 3 | 8 | 0 | 14 | 5 | 6 | 10 | 2 | 12 | 31 |
| | 1.9 | 1.5 | 3.3 | 10.0 | 2.4 | 6.7 | 10.0 | 5.0 | 4.4 | 7.5 | 17.5 | 28.4 |
| B. bimaculatus | 10 | 11 | 14 | 15 | 4 | 19 | 4 | 5 | 8 | 10 | 18 | 38 |
| | 3.0 | 3.3 | 6.3 | 19.3 | 2.7 | 10.4 | 14.2 | 6.0 | 3.8 | 17.5 | 30.1 | 39.6 |
| B. impatiens | 7 | 13 | 22 | 49 | 4 | 31 | 9 | 12 | 3 | 9 | 13 | 23 |
| | 3.0 | 2.3 | 9.7 | 39.4 | 2.2 | 18.9 | 21.9 | 6.5 | 2.6 | 28.3 | 24.5 | 36.6 |

*G is significant at 245; P=.05, 44 degrees of freedom.

**The upper number in each row is the number of observed visits to each particular flower type.

++The lower number in each row is the expected number of visits to a flower type based on the flower type density within each plot.

TABLE XXVIII

G-TEST OF THE ATTRACTIVENESS OF THE FLOWER TYPES TO NON-BUMBLEBEE POLLINATORS*

| Pollinator | Flower Type W-2/3 | F-1/2 | P-1 | P-2/4/6 | P-3/5 | R-1 | R-2 | R-3** |
|---|---|---|---|---|---|---|---|---|
| Silver-spotted skipper | 17 | 5 | 20 | 1 | 10 | 1 | 6 | 10++ |
| | 3.3 | 10.5 | 5.9 | 7.7 | 5.0 | 4.8 | 14.2 | 16.7 |
| Small Bees | 17 | 16 | 10 | 3 | 8 | 12 | 18 | 25 |
| | 5.2 | 19.4 | 5.9 | 8.3 | 6.3 | 12.9 | 24.2 | 25.2 |
| Vespula spp. | 10 | 35 | 12 | 5 | 10 | 8 | 6 | 34 |
| | 4.0 | 37.5 | 9.8 | 9.7 | 9.4 | 9.9 | 19.5 | 30.1 |
| Other | 7 | 6 | 7 | 0 | 2 | 6 | 5 | 4 |
| | 1.7 | 7.1 | 2.4 | 2.9 | 1.2 | 6.0 | 9.9 | 7.4 |

*G is significant at 140; P=.05, 44 degrees of freedom.

**In this analysis certain flower types were condensed to increase the expected values.

++The upper number in each row is the observed and the lower number in each row is the expected number of pollinators to visit a flower type based on the flower type density within each plot.

TABLE XXIX

CHI SQUARE ANALYSIS OF THE EFFECT OF TIME OF DAY ON POLLINATOR ACTIVITY (RAW DATA)*

| Insect | Time of Day 8/9 | 10/11 | 12/1 | 2/3 | 4/5 | 6/7 | Total |
|---|---|---|---|---|---|---|---|
| Bumblebees | 112 | 86 | 89 | 180 | 226 | 119 | 812 |
| | 88.4 | 78.2 | 108.2 | 202.5 | 241.2 | 93.6 | |
| Silver-spotted skipper | 2 | 8 | 13 | 26 | 20 | 1 | 70 |
| | 7.6 | 6.7 | 9.3 | 17.5 | 20.8 | 8.1 | |
| Small Bees** | 6 | 9 | 26 | 24 | 40 | 4 | 109 |
| | 11.9 | 10.5 | 14.5 | 27.2 | 32.4 | 12.6 | |
| Vespula spp. | 1 | 4 | 20 | 47 | 44 | 4 | 120 |
| | 13.1 | 11.6 | 16.0 | 29.9 | 35.6 | 13.8 | |
| Total | 121 | 107 | 148 | 277 | 330 | 128 | 1,111 |

*The upper number in each row is the observed and the lower is the expected. $X^2 = 93$; significant at .05, 15 degrees of freedom.

**Andrenidae and Halictidae.

TABLE XXX

CHI SQUARE ANALYSIS OF THE EFFECT OF TEMPERATURE ON POLLINATOR ACTIVITY (RAW DATA)*

| Insect | Temperature (° C) 14.4-18.3 | 19 | 20.3 | 21.2 | 22.5 | 23.6 | 24.4-27.8 | Total |
|---|---|---|---|---|---|---|---|---|
| Bumblebees | 182 | 119 | 112 | 83 | 104 | 158 | 54 | 812 |
| | 140.3 | 102.3 | 101.6 | 84.9 | 124.3 | 187.1 | 72.4 | |
| Silver-spotted skipper | 1 | 6 | 5 | 8 | 16 | 23 | 11 | 70 |
| | 12.1 | 8.8 | 8.8 | 7.3 | 10.7 | 16.1 | 6.2 | |
| Small Bees** | 7 | 7 | 14 | 19 | 19 | 32 | 11 | 109 |
| | 18.8 | 13.7 | 13.6 | 11.3 | 16.7 | 25.1 | 9.7 | |
| Vespula spp. | 2 | 8 | 8 | 5 | 31 | 43 | 23 | 120 |
| | 20.7 | 15.1 | 15.0 | 12.4 | 18.4 | 27.7 | 10.7 | |
| Total | 190 | 140 | 139 | 115 | 170 | 256 | 99 | 1,111 |

*The upper number in each row is the observed and the lower is the expected. $X^2 = 128$; significant at .05, 18 degrees of freedom.

**Andrenidae and Halictidae.

# VITA

Clinton Victor Kellner was born in Berkeley, California (August 8, 1953) and lived in San Lorenzo, California. Both of these cities are in the San Francisco Bay Region. After graduation from Arroyo High School, he attended the University of California at Berkeley where he received the Bachelor of Arts degree in the ecology plan of the Biology major in December 1975. During his undergraduate career the writer worked as a laboratory aide for the U. S. Forest Service Fire Chemistry Laboratory and for the U.C.B. Environmental Physiology Laboratory. He also worked as a gardener for the U.C. Botanical Garden and as a curator of an insect collection for Dr. E. I. Schlinger.

After graduation the writer married Madeline Radkey, from Berkeley, who was pursuing a Master's degree in Public Health Nutrition at The University of Tennessee, Knoxville. Subsequently, the author enrolled in the Graduate Program in Ecology at The University of Tennessee, Knoxville. His interest in plant-insect interactions led him to the study of the pollination ecology of the azalea hybrid swarm on Gregory Bald. Upon receipt of the Master's degree the author will enroll in the Graduate Group in Ecology at the University of California, Davis and pursue a doctoral degree in herbivore-plant interactions.